2. ENVIRONMENTAL CHEMISTRY & TECHNOLOGY

2.1. Lectures

L02 DEGRADATION PRODUCTS OF SYNTHETIC POLYMERS AS EMERGING ENVIRONMENTAL CONTAMINANTS

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Introduction

Synthetic polymers belong to attributes of modern life; it is hard to imagine contemporary lifestyle without them.

These materials came into existence before almost 200 years. In 1811 french pharmacist Henry Braconnot prepared the first man-made material by treating of wood and cotton by concentrated nitric acid. This material was called "xyloidin" and it hasn't found any practical use. The term "polymer" was proposed later in 1833 by Jöns Jakob Berzelius. The first semi-synthetic polymer, vulcanized rubber, was synthesized by Charles Goodyear in 1839. Very popular and widely used celluloid was discovered in 1870 by John Wesley Hyatt; it was prepared from nitrocellulose and camphor. All these polymers were based on raw materials of natural origin. The first fully synthetic (and also very popular) polymer was bakelite, which was prepared between years 1907–1909 by Belgian chemist Leo Baekeland.

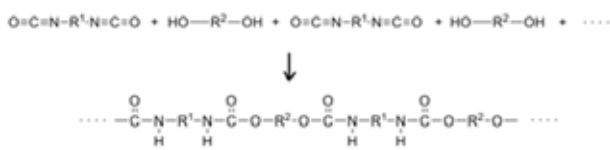

Fig. 1. **Polyurethane synthesis**

In our study we focused our attention on polyurethanes. Thesse polymers were discovered in 1937 by german chemist Otto Bayer. Their synthesis is quite simple; by reaction of diisocyanate with diol the polymeric chain is formed containing both monomer units connected by urethane bonds:

Toluene diisocyanate is most often used raw material; if polyols are used instead of diols then 3-dimensional and more firm polymer network is formed.

Nowadays, polyurethanes are very popular and widely used due to their extremely flexible properties, which can be "tailored" with respect to their planned use. Flexible low density foams (6 kg m^{-3}) are used in bedding and upholstery, semi-rigid ones serve as packaging foams and rigid are used as insulation foams. High density foams (400 kg m^{-3}) in flexible version serve as footwear midsoles and outsoles, rigid ones are used as integral skin in vehicle interiors and rigid foams are produced as simulated wood. Microcellular foams and elastomers (density 800 kg m^{-3}) are used for fabric coatings and synthetic fibers production and also for vehicle facia and other exterior parts of cars and also as structural foam. Solid polyurethane elastomers - RIM (density 1,200 kg m^{-3}) are used for example in production of printer rolers.

The worldwide production of polyurethane is constantly increasing and in 2003 it overcame 10 million of tons. From this amount 1/3 is produced in Europe, another 1/3 in North America and the last 1/3 in the rest of the World[1].

The mass flow of polyurethanes in Europe in 2000 was described by Ron Zevenhoven[2]. The total PU consumption was approx. 3 Mt yr^{-1} (cca 1.8 Mt yr^{-1} of PU foams, cca 0.8 Mt yr^{-1} of rigid foams, cca 0.4 Mt yr^{-1} of RIM and elastomers). From this amount only cca 150 kt yr^{-1} is recycled and approximately the same amount is incinerated. Remaining cca 90 % of the amount produced end in landfills, where it can undergo various decomposition reactions. Therefore, it would be useful to enhance biodegradability of these materials; on the other side, the products of decomposition could be environmentally dangerous.

In our study we focused our attention on the identification of degradation products of polyurethane with enhanced biodegradability. Either natural conditions or simulatend ageing were used, both volatile and non-volatile compounds were analysed.

Experimental

Polyurethane foams (PUFs) were prepared at the Institute of Material Science by reaction of diisocyanate and polyol; part of the synthetic polyol (up to 10 %) was replaced by biodegradable biomass originated polyol (cellulose acetate, wheat protein, acetylated potato starch, carboxymethylcellulose, 2-hydroxyethylcellulose). In parallel, control PUF was prepared by standard procedure (i.e. without addition of biomass polyol).

Hydrolytic Degradation

3 g of PUFs were refluxed with 150 dm^3 of deionized water for 8 hours. Leachates were analyzed by LC/ESI-MS.

Natural Photodegradation

Flat pieces of PUFs were placed into flower window box on the top of soil layer and fixed by small stone. These pieces were let outside for 2 months. After this period the PUFs were extracted by ultrasonication in acetonitrile or n-hexane; acetonitrile extracts were analysed by LC/ESI-MS, hexane extracts by GC/MS.

Accelerated Ageing – Detection of Non-Volatile Compounds

Flat pieces of PUF were placed under the UV-discharge tube and irradiated for 6 hours. Exposed PUFs were sonicated in *n*-hexane, the extract was volume reduced and analyzed by GC/MS.

Accelerated Ageing – Detection of Volatile Compounds

Flat pieces of PUFs were placed into accelerated ageing device (Fig. 2.) composed of quartz tube open on one end (length 24.5 cm, I.D. 3.5 cm) with input tubing and Teflon cover. The device was placed under UV discharge tube. Two SPME devices were inserted into both input and output tubing (the first with polydimethylsiloxane fibre, the second with polyacrylate fibre) and the ends of the device together with SPME holders were covered by aluminium foil to protect them against the UV light. The irradiation tooks place for 6 hours. After this period, SPME devices were directly analyzed by GC/MS.

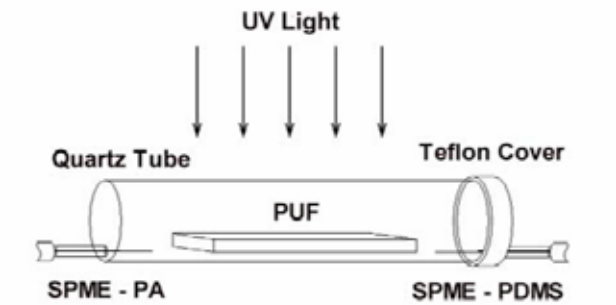

Fig. 2. **Accelerated ageing device**

HPLC/MS

For HPLC/MS the Esquire-LC instrument (Bruker Daltonics, Bremem, Germany) was used. This system consists of the Agilent HPLC 1100 Series with binary gradient pump, electrospray ion source and spherical ion trap analyzer. A Supelcosil™ LC-18DB column (2.1 × 250 mm, 5 μm particles) was used (Supelco, USA). Gradient elution from 30 to 100 % acetonitrile in water in 30 min was used at constant flow rate of 0.25 ml min^{-1}. For the detection both UV-VIS detector of DAD type, and mass spectrometry were used. Drying temperature in electrospray was 350 °C, nebuilizing gas (N_2) pressure was 50 psi, and drying gas (N_2) flow was 14 dm^3 min^{-1}. Both positive and negative ions were registered (in separate runs).

GC/MS

System Agilent 6890N GC/5973 MSD (Agilent Technologies, Waldbronn, Germany) was employed. The column was HP-5MS 5 m × 0.25 mm × 0.25 μm, helium at a flow of 1 ml min^{-1} was used as carrier gas in constant flow mode. Temperature program was as follows: 50 °C for 1 min, then to 280 °C at 5 °C min^{-1}, final isotherm 5 min. 1 μl of sample was injected in splitless mode at a temperature of 280 °C with splitless time of 1 min. Interface temperature was 260 °C, temperature of ion source and quadrupole 230 and 150 °C, respectively. Electron ionization at 70 eV electron energy was used, spectra were registered in scan mode within the range of 50–450 amu. NIST 05 spectral library was used for the identification of separated compounds.

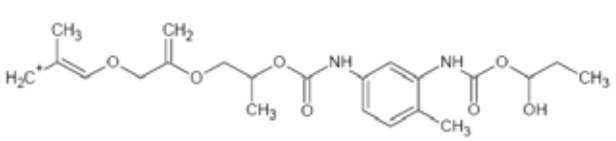

Fig. 3. **Structure of PUF hydrolytic degradation product**

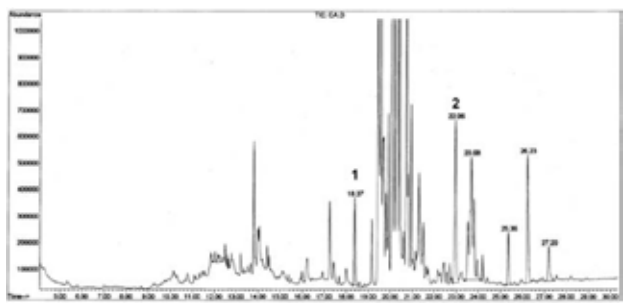

Fig. 4. **PUF non-volatile degradation products. Compound identification: 1: bis(2-ethylhexyl) ester of hexanedioic acid; 2: di-tolyl-isocyanate**

Results

Typical LC chromatogams of PUF extract show only two peaks at the beginning. Using the LC/MS/MS experiments and MS^n with direct insfusion of the sample, the probable structure of main degradation product was proposed (see Fig. 3.).

This structure is evidently a fragment of polyurethane polymer chain.

Fig. 4. shows the chromatogram of hexane extract of polyurethane after UV irradiation. Using the database search several peaks were successfully identified, but in many cases the identification was unsuccesfull, in spite of the fact that the experimental spectrum was of good quality. The most probable explanation is that the NIST05 database doesn't contain

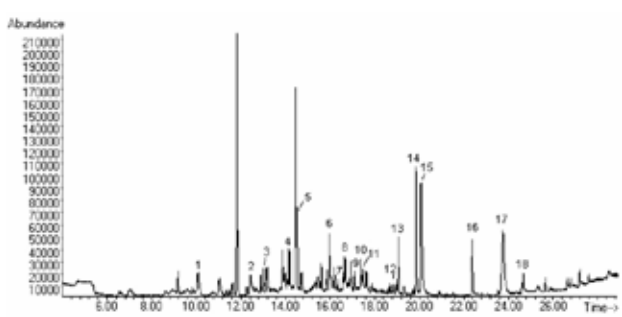

Fig. 5. **Volati1e degradation products (PDMS fibre). Compound identification: 1: 2-methyl-1,3-dioxane; 2: 2,6-diisocyanatotoluene; 3: 6,10-dimethyl-5,9-undecadiene-2-one; 4: 2,5-di-terc-butyl-1,4-benzochinone; 5: pentadecane; 6: hexadecane; 7: 2,6-bis(1,1-dimethyethyl)-4-(1-oxopropyl)phenol; 8: dodecanoic acid methylester; 9: 4-decyl-morpholine; 10: heptadecane; 11: 2,6,10,14-tetramethyl-pentadecane; 12: ?? (isoprenoid alkane); 13: tetradecanoic acid isopropylester; 14: 4-undecyl-morpholine; 15: N,N-dimethyl-1-hexadecanamine; 16: 4-tetradecyl-morpholine; 17: squalene; 18: 4-hexadecyl-morpholine**

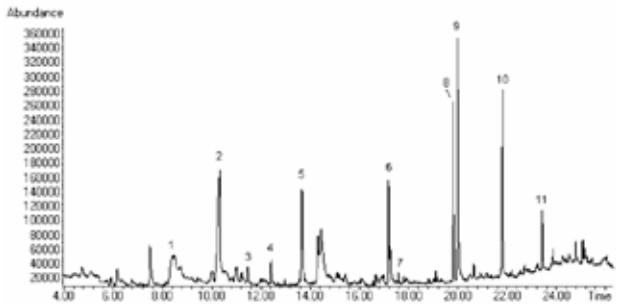

Fig. 6. **Volati1e degradation products (PA fibre). Compound identification: 1: 2-(2-ethoxyethoxy)-ethanol; 2: 2-ethylhexanoic acid; 3: 1,3-dioxane; 4: 2-methyl-1,3-dioxolane; 5: 2-methyl-1,3-diisocyanatobenzene; 6: 2,6-bis(1,1-dimethyethyl)-4-(1-oxopropyl)phenol; 7: 4-decyl-morpholine; 8: 4-undecyl-morpholine; 9: N,N-dimethyl-1-hexadecanamine; 10: 4-tetradecyl-morpholine; 11: 4-hexadecyl-morpholine**

mass spectra of compounds of this type. The only possibility of structure revealing in these cases remains the manual interpretation using the common fragmentation rules[3].

Fig. 5. shows the chromatogram of volatile degradation products of PUF with addition of hydroxyethylcellulose adsorbed by SPME on PDMS fibre. Various classes of compounds were found – alkanes, esters of fatty acids, morpholine derivatives, but also poisonous 2,6-diisocyanatotoluene. Fig. 6. depicts the same degradation products adsorbed on polyacrylate fibre. In this case more polar compounds are preferred like morpholine derivatives, but again toxic 2,6-diisocyanatotoluene (compound 2) was found.

Composition of the degradation products also depends on the type of biodegradable filler. Toluene diisocyanate was found only when hydroxyethylcellulose and cellulose acetate were applied.

Conclusions

Degradation products of polyurethane foams with enhanced biodegradability were studied. Both degradation under natural conditions and accelerated ageing were used, both non-volatile and volatile compounds were analysed using liquid and gas chromatography interfaced to mass spectrometry. The composition of degradation products was influenced by the type of biodegradable filler used instead of 5 % of polyether polyol in the polyurethane synthesis. When modified cellulose was used, poisonous toluene diisocyanate was identified as volatile degradation product. The identification of products separated by GC by library search was unsuccesfull in many cases; the reason is problably caused by the fact that the used MS library is commonly oriented and doesn't contain the spectra of PUF degradation products.

Following research will be focused on the quantitative analysis of PUF degradation products, either using SPME and suitable surrogates, or using adsorption tubes with suitable adsorbent.

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L03 USAGE OF GAS CHROMATOGRAPHY AND IMS DETECTION FOR EVALUATION OF POLYMER BARRIER MATERIAL PROPERTIES

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Introduction

The protective properties are the most important parameters for selection of well-suited materials for construction of individual protective equipment (IPE). These properties determine ability of a given IPE against the highly toxic agents and industrial harmful substances under short time and/or long time contamination in the gaseous and liquid phases.

The most frequently used protective materials for IPE are polymer barrier materials. Their numerous advantages such as easy availability, possibility of mass production, relative low cost as well as the broad range of manufactured qualities determine their use for different protective purposes. Single-layer or laminated polymers and textile with polymeric or elastomeric layer are used as barrier materials. It is possible to use metallic film, PET sheet with layer of SiOx (e.g. material CERAMIX from company ALCAN) or other nanolayers, adsorption textile (e.g. SARATOGA with spherical sorbent or Charcoal Cloth made from activated carbon fibres) eventually special membranes (e.g. NAFION, GORE-TEX, POROTEX, PURTEX etc.) with specific diffusion properties for toxic agents.

Resistance of polymer barrier materials against harmful substances is defined as parameter called breakthrough time (BT)[1,2,3,4]. Breakthrough time is the most widespread way of barrier materials evaluation in term of constructional usage of materials, their manufacture and conditions of their selection.

Experimental

The procedures for evaluation of protective properties are elaborated in accordance with the Czech Technical Standard ČSN ISO EN 6529 (October 2001) which results from American Standard ASTM F 739-99a (August 1999)5,6. The above-mentioned standard describes experimental methods used for testing of barrier materials resistance against permeation of liquid and gaseous substances and, among other things (e.g. conditions of measurement for closed-loop or open-loop, continual and discontinual measurement, preparation of samples etc.) recommends suitable analytical techniques for evaluation of permeation toxic vapors. The gas chromatography (GC) is one of these techniques which is commonly used for detection and identification of chemical warfare agents (CWA) as well as ion mobility spectrometry (IMS)7,8,9,10. The 2, 2'-dichlorodiethylsulfide (sulphur mustard, HD) was used for measurement of permeation as testing chemical. The double-sided butyl rubber polyamide fabric was used as the used tested material.

Permeation Method

The following methods were selected for evaluation of barrier properties of polymer materials11,12,14,15. The principle of these methods is illustrated by visual demonstration on Fig. 1. where the scheme of alternative permeation cell that is used for measurement at aerodynamics conditions. The alternative permeation cell respects requirements of standard ČSN EN ISO 6529. This cell is made from stainless steel. The clamping system is solved by the one central withdrawal nut (see scheme on Fig. 1.) that makes possible not only quick clamping and exchange of tested materials but also total obturation of samples. The tested material separates the alternative cell into two parts. Upside of cell contains testing chemical (here sulphur mustard) and on the other side the permeating gas or vapor is swept away into the carrier gas leading to detector. The carrier gas and agent vapors mixture is then analyzed by suitable detection techniques.

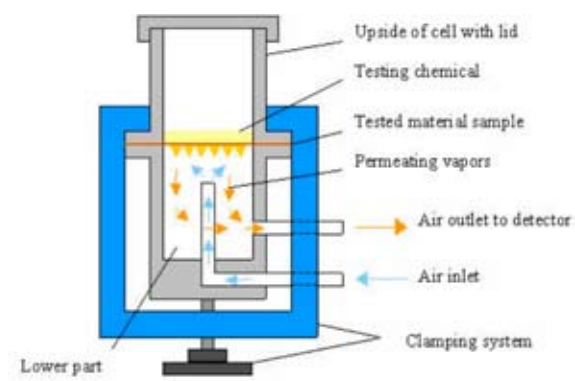

Fig. 1. **Scheme of alternative permeation cell used for measurement at aerodynamics conditions**

Composition of Experimental System

The experimental system for permeation measurement of toxic agents was designed in accordance with the standard ČSN EN ISO 6529. The clean air comes through drying column (the clean air is represented by blue arrows) washes lower part of tested material. The air stream carry away permeating vapors of testing chemical to the given detector (the air containing vapors of testing chemical is represents by red arrow). This system was modified according to the used kind of detection (see scheme on Fig. 2.).

GC and IMS detector GID-3 were used for analysis of permeating sulfur mustard vapors. The GC equipped with FID detector, Agilent 6890, was used for separation of gases and vapors between mobile and stationary phase. The components are separated on the basis of holding ability of the stationary phase into the chromatographic column. This analytical method enables evaluation of taken samples using

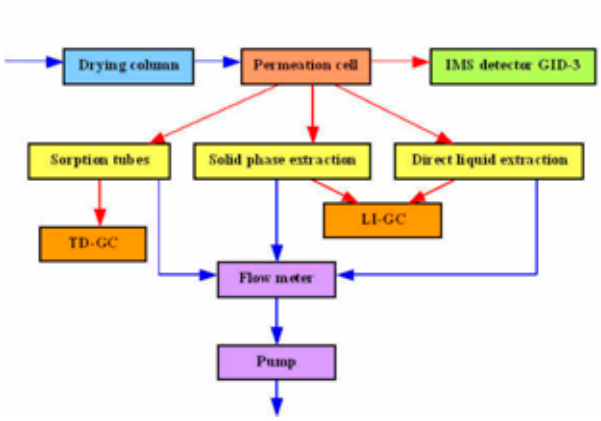

Fig. 2. **Composition of usable experimental system for measurement of permeation of toxic agent through protection barrier materials**

thermal desorption from solid phase sorbent (Tenax™ TA), direct solvent liquid extraction (n-hexane) and solid phase extraction (SPE) method. The IMS detector GID-3 operates on the basis of the different mobility of ions in gaseous phase in the homogeneous electric field.

The investigated barrier material sample was fixed inside the alternative permeation cell (see scheme on Fig. 1.) and contaminated from the upper side with defined amount of liquid HD agent. The permeating agent vapors were sampled from the permeation cell space under the material sample by the IMS detector. The air-drying column was connected at the front end of the permeation cell inlet to protect IMS detector against moisture. IMS detector was connected to the PC where the measured data was continually acquired, saved and graphically evaluated (see chart on Fig. 3. and Fig. 4.) with using the software BarieraSW2006 especially designed for this purpose.

The scheme of LI/GC and TD/GC testing system is shown on Fig. 2. The same configuration of permeation cell including the investigated material sample placing and contamination procedure was used. The special sampling device equipped with a controlled air pump and flow meter was used for permeating agent sampling by the solid phase extraction ASSET-32™ tubes, direct solvent liquid extraction (DSLE) by n-hexane (for GC, ≥99.0 %) and DAAMS Tenax™ thermal desorption tubes. Air flow of 100 mL/min was set up. Agilent GC equipped with FID detector was used for analysis of liquid sample.

Results

The results of sulphur mustard permeation through tested materials obtained by IMS detector and GC (thermal desorption, direct liquid extraction and solid phase extraction) are compared in the chart on Fig. 3. and Fig. 4. Observed concentration shift shown in the chart is probably caused by different sensitivity of used methods. SPE method is more sensitive than direct solvent liquid extraction and direct solvent liquid extraction method is more sensitive than IMS method.

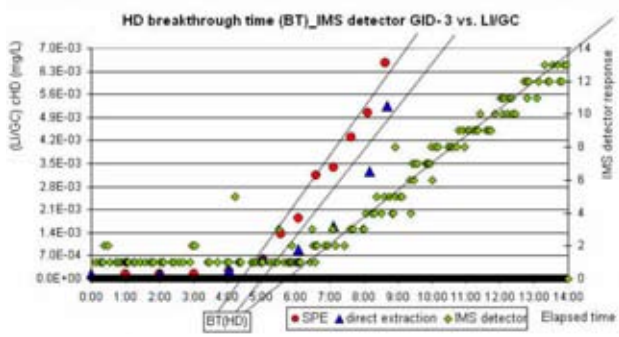

Fig. 3. **he IMS and LI/GC techniques (direct solvent liquid extraction and solid phase extraction) results comparison**

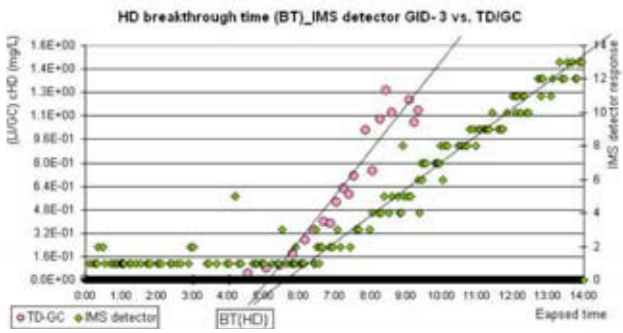

Fig. 4. **The IMS and TD/GC techniques (thermal desorption) results comparison**

Breakthrough time of HD was determined on the basis of graphical analysis of permeation curves called time-lag. The permeation curve was interlayed by line13–19. The point where the line is crossing the x-axis represents the HD breakthrough time of investigated material (see Table I).

Table I
The values of HD breakthrough time obtain time-lag method

The used techniques	Breakthorough time of HD minutes	hours
IMS	350	5 : 50
TD-GC	310	5 : 10
LI-GC (SPE)	270	4 : 30
LI-GC (DSLE)	300	5 : 00

Basically the breakthrough time is dependent also on the other factors then the used analytical method. Temperature is one of the most important factors, which strongly influences the permeation process. The following experiments, held under the same conditions, have been performed to quantify the temperature dependence. The course of permeation was monitoring by the IMS detector GID-3, which is preferable for long continual measurement. The measurement was proceeded in temperature range from 15 °C to 40 °C by 5 °C. The lower range limit was defined by freezing point of sulphur mustard, which is 14.5 °C. The upper limit was restricted by

tolerable ness of protection means. The fatal overheating of human body can happen at the temperatures over 40 °C.

The charts of all permeation curves are shown on Fig. 5. The permeation of HD agent through polymer membrane is faster with increasing temperature and permeation will be sharper course.

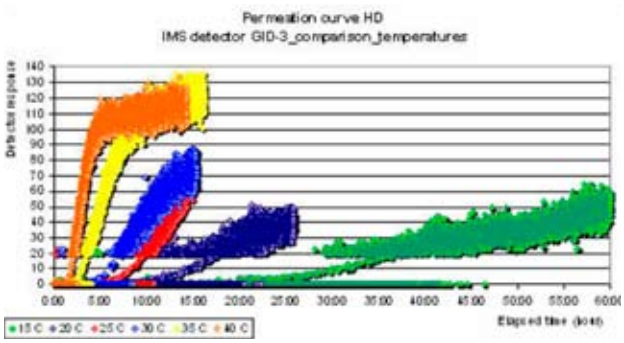

Fig. 5. **The comparison of permeation curves obtained at different temperatures**

The related values of breakthrough times evaluated with using time lag method are presented in Table II.

Table II
The values of HD breakthrough time obtained with using the time-lag method for measurement under various temperatures by IMS detector

Temperature	Breakthorough time of HD	
	minutes	hours
15 °C	1485	22 : 20
20 °C	600	12 : 00
25 °C	440	7 : 20
30 °C	285	4 : 45
35 °C	150	2 : 30
40 °C	90	1 : 30

The dependence of HD breakthrough time on temperature is plotted in chart shown on Fig. 6. The points in the chart were interlaced by exponential curve and this curve was expressed by regression equation. The exponential equation can be used for breakthrough time on temperature dependence quantification and the parameters could be used for different materials resistance comparison.

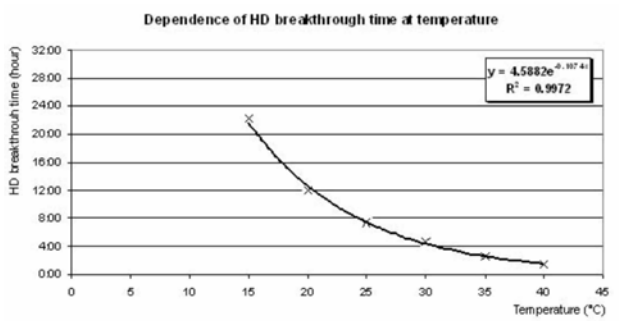

Fig. 6. **The chart of HD breakthrough time dependence on the temperature**

Conclusions

The main purpose of this measurement was to compare the analytical methods and/or detection techniques used for the breakthrough time's evaluation. On the basis of obtained results comparison it is possible to submit that the value of HD breakthrough time is strongly dependent on sensitivity of used analytical method. The IMS detector enables to perform the continual measurement of permeation HD in real time. The result is permeation curve represented by increase of permeating vapors amount in time. The GC method providing an exact determination of kind and amount of permeating compound and products of interaction between tested polymer and permeating toxic agent, i.e. qualitative a quantitative analysis in contrast to IMS. However, the GC method does not enable to perform the continual measurement and is not so suitable for long term measurement. Otherwise, the IMS method indicates course of permeation vapor through polymer membrane but is not able to determine kind and amount of permeated compound.

But simultaneous use of both detection methods used simultaneously enable to perform the more exact definition of tested polymer barrier material character.

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L04 COMPLEX ESSESSMENT OF ORGANOPHOSPHATES LOW DOSE CHRONIC EXPOSURE ON ENDOTHELIUM, MACROPHAGES, PLATELETS AND ESTERASES

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Introduction

Morphofunctional disturbances of circulation in rat embryos intoxicated by derivatives of organophosphates (OP), a role of endothelium directly affected by OP under acute intoxications[1,2], and various data on the role of endothelium in development of peripheral neuropathies of various genesis[3,4,5] were the basis for consideration of endothelium as one of the main targets under chronic intoxication with OP. In this research work, an attempt has been undertaken to clarify an impact of circulatory disturbances, the cellular component of haemostasis, and neuropathy target esterase (NTE) activity upon reaction of rat organism under intoxication with low doses of OP.

Materials and Methods

In these experiments diisopropylfluorophosphate (DFP), which can induce the delayed polyneuropathy, and paraoxon which has no such effect, were applied with drinking water at doses 10^{-2} mg kg^{-1} (1/100 LD_{50}) and 10^{-4} mg kg^{-1} (1/10,000 LD_{50}). The intoxication was conducted daily 5 times in a week for 3 months. Esterases of choline and non-choline substrate specificity[6,7,8,9] were studied; for rat brain, the enzyme activity was calculated per mg of protein[10]. To investigate the OP effects upon NADPH-oxidase system, we used the functional state of peritoneal macrophages with fluorescent microscope and fluorescent probe dichlorfluorescein diacetate (DCF)[11]. Kinetic parameters of platelet aggregation were studied by low angle light scattering technique[12]. The functional activity of endothelium under OP intoxication was studied with rat aorta by method of endothelium-dependent relaxation[13,14]. The results obtained were processed by variation statistics and MS Excel software.

Results and Discussion

Comparing the level of inhibition of esterases in blood plasma after 3 months' intoxication with the OP, we have found that the residual activity of NTE was much lower than that of red blood cells' acetylcholine esterase (AChE). Inhibition of NTE activity in rat brain 2 months after stopping intoxication with DFP gives an indirect evidence for development of neurotoxic effects. Activity of esterases after chronic intoxication demonstrates a switch of inhibition from one enzyme to another, embracing both choline specific and non-specific esterases. It is noticeable that the most prolonged neurotoxic effect (20–30 % reduction of NTE activity after 6 months' period of the post-intoxication recovery) remained in the platelet-rich plasma (PRP).

The next method demonstrating its efficacy for diagnostics of the low dose intoxication with OP was estimation of the level of generation of reactive oxygen species (ROS) during "respiratory burst" by professional phagocytes (macrophages). It is a widely known fact that macrophages

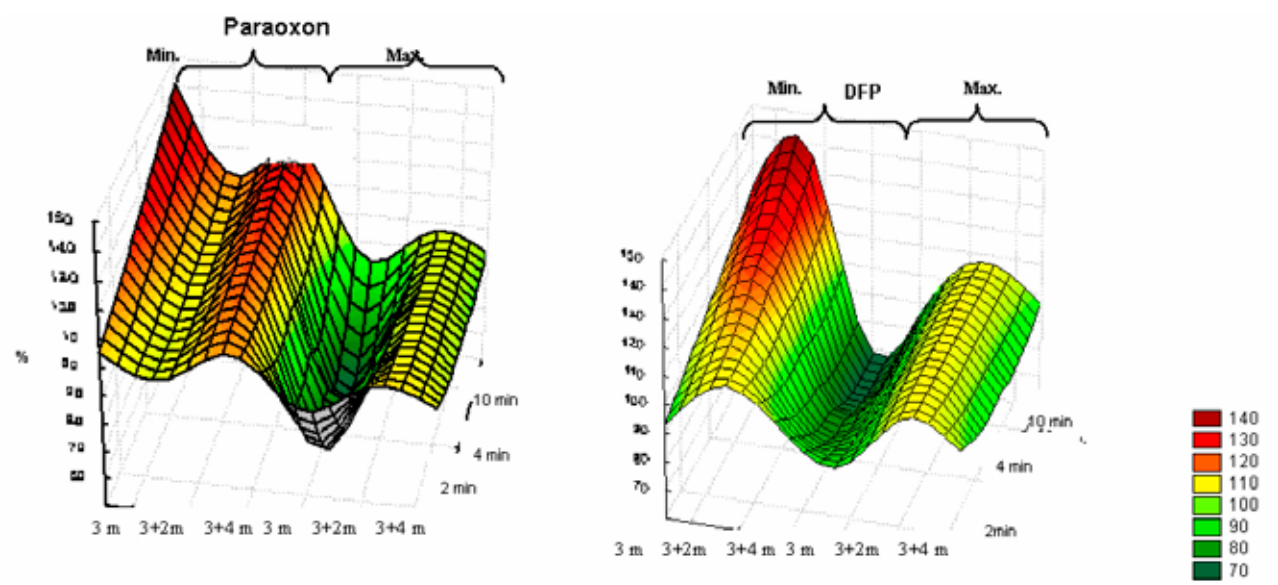

Fig. 1. **Generation of ROS by mice macrophages after 3 months' chronic intoxication and 2 and 4 months after cessation of the intoxication with paraoxon and DFP at doses 10^{-4} mg kg^{-1} (min) and 10^{-2} mg kg^{-1} (max)**

are not only scavengers and alarm cells but they also have important functions to occupy key positions in every kind of immune response: production of antibodies, induction of cell immune reactions, development of immunologic memory and tolerability, thus correctly being named "dispatcher cells". In our experiments, the functional activity of mouse macrophages was activated under intoxication with OP at 10^{-4} mg kg^{-1} (Fig. 1.). Activation of phagocytes affected by low doses of OP leads to production of ROS (hydrogen peroxide, superoxide anion, singlet oxygen and hydroxyl anions), which cause modification of tissue and serum proteins and lipids to further cause their obtaining of antigenic properties. Thus, activation of phagocytes is an autocatalytic process and can lead to formation of the vicious circle. On the other hand, absence of macrophages' activation under exposure to large doses of OP indicates a suppression of antigen-presenting function of these cells; it is also known that chemical agents inhibiting this function in most cases cause suppression of immune response[16].

The animals' immune status was further tested with low angle light scattering technique and studies of functional activity of blood platelets. Besides of a leading role in haemostasis, blood platelets play also an important role in immune reactions being a mediator between these two physiologic systems[17,18]. For the expense of binding with C1q receptor, the platelets can adhere to endothelium of capillaries, where local interaction of platelets with activating agents can take place[19,20].

Peroral chronic administration of DFP and paraoxon to laboratory animals for 3 months with the following examination at 2, 4 and 5 months after cessation of the intoxication demonstrated that in both cases a pronounced disturbance of the functional activity of platelets was observed, followed by a prolonged period of recovery of kinetic parameters of platelet aggregation (Fig. 2.). In 3 months of chronic intoxication with DFP a marked increase of EC_{50} was registered, similar to the case with Russian VX[21]. In contrast to DFP and RVX, paraoxon did not have a visible effect on sensitivity of platelets, but affected the maximal velocity of aggregation to the greater extent.

Investigation of blood vessel endothelium revealed that

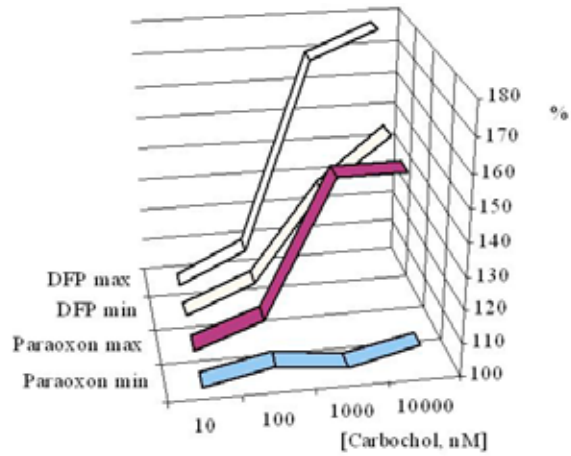

Fig. 3. **Relative excess over control (100 %) of the blood vessel contracting force in 3 months of intoxication with DFP and paraoxon at doses 10^{-2} and 10^{-4} mg kg^{-1} (carbachol was administered at the background of pre-constriction with norepinephrine)**

under chronic intoxication with OP an inhibition of endothelium-dependent relaxation occurs with both DFP and paraoxon, though the former was more potent at the same doses (Fig. 3.). Moreover, the level of pre-constriction with epinephrine under exposure to DFP was significantly different from that observed under exposure to paraoxon. So not only the level of relaxation following pre-constriction did change but also the background tonus of blood vessels and dynamics of the pre-constriction *per se*. In 2 months after cessation of the intoxication, inhibition of the endothelial function was nevertheless rather pronounced in both groups of rats that were exposed to DFP and paraoxon at 10^{-2} mg kg^{-1}. In 5 months, there were significant changes only in rats exposed to DFP, being at the same level in both groups of intoxicated rats administered to DFP at doses 10^{-4} and 10^{-2} mg kg^{-1}.

Conclusions

Esterases of various geneses, localization and functional specialization should be molecular targets for OP.

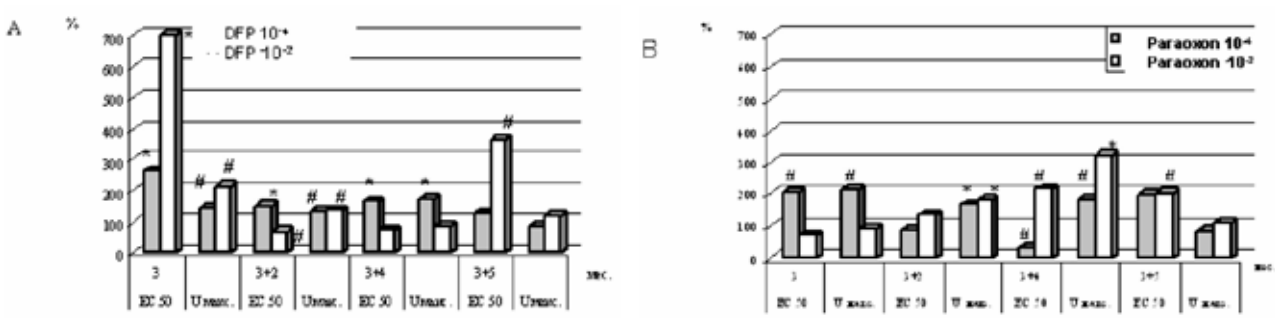

Fig. 2. **Relative changes of the medium effective concentration of activator (EC50) and maximal velocity of platelet aggregation (Umax) after peroral administration of DFP (A) and paraoxon (B) at doses 1×10^{-2} (max) and 1×10^{-4} (min) mg kg^{-1}: after 3 months of intoxication, then at 2, 4 and 5 months of the "recovery period"; (# – $P < 0.1$; $^{*}P < 0.05$)**

Changes in activity of esterases under prolonged exposure have a phase character with predominant inhibition of NTE as a delayed effect.

Endothelium should be one of the main tissue targets in case of chronic low dose intoxication with OP.

Blood platelets and phagocytes could be sensitive testing models to estimating effects of the low level exposure to OP.

The pathogenesis of low dose chronic intoxication with OP is probably based upon the two complementary factors: development of immunoreactivity and tissue/circulatory hypoxia.

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L05 INFLUENCE OF CLIMATE CONDITIONS AND AIR CONTAMINATION ON VILLAGE INHABITANTS HEALTH

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Introduction

The understanding of health as a main component of environment quality accrues from the Agenda 21 concerning the right of human beings to healthy and productive life[1]. Air, one of the components of environment, is polluted primarily by anthropogenic factors. Generally, the countryside is considered a healthy environment[2]. However, there are very few studies to prove this assumption since the air monitoring is a long-term and expensive method and therefore carried out only on a few monitoring points[3].

The aim of the study was to assess the state of climatic conditions and state of air quality in relation to the rate of diseases among the inhabitants of Královce-Krnišov village by a simple method, based on a comparison of potential conditions of contamination with real state of population health. The Southern Sitno micro region is important for tourism development and geographical conditions.

Experiment and Methods

Using the reconnaissance of the place was carried out terrain research. Information and sources of contaminants were observed together with the perception of problems related to air quality and environmental health. The obtained data were further processed by the statistical method of contingential tables, on the basis of which the individual mutual relations were evaluated, at the free scope degree of 1 and the level of importance 0.01 (χ quadrate). An assessment of the state and movement of air required the analysis of the relief as well as sunshine input as well as the regional state and movement of air for which GIS tools (geomedia) were used. Contamination was interpreted from the map of the secondary landscape structure and sources of contaminants. The relation between movement and quality of air and environmental health was worked out on the basis of the basic data parametric analysis with the subsequent table synthesis and mathematic statistics

Results and Discussion

On the territory of the Kráľovce–Krnišov village (the Štiavnické Mountains) cold, mild warm and warm mountain climate prevails. The average yearly temperature spans are from 5.5 °C to 8 °C. The average cloud amount is around 62 %. The number of sunny days is around 48 a year and the number of cloudy days is around 125. The territory is characterized by regional northern, north-west air circulation with a low year and day amplitudes prevailing all the year round. There are frequent temperature inversions during the radiation weather with a depression circulation. There is also an intensive effect of a "temperature island" with a distinctive increase of day amplitudes supported by the effect of winds of low intensity. Negative influences on environmental health are diseases caused by contamination from regional emissions resulting from the type of climate and skin diseases as a consequence of contamination of anthropogenic and natural factors – frequent and concentrated air circulation especially in winter time. Frequent skin diseases and flu are typical for inversion type of weather. Respiratory diseases and diseases related to increased stress conditions due to the amplitudes changes, such as blood pressure disorders, stress and cardiovascular diseases, were confirmed. The increase of the all above diseases was statistically confirmed.

The territory of the Southern Sitno micro region is exposed to the influence of industry confirmed by the considerable damage of the environment by pollutants distributed in conditions of mesoclimate. The contamination of the regional type concerns mainly average annual NO_2 and SO_2 concentrations. The contamination in the settlement is therefore of integrated character from the following sources:

- *transport related emissions* – the contamination intensity is lower in winter and spring season, no statistical importance with any disease was shown;
- *heating related emissions* – at inversion type of weather there is an increased intensity of contamination especially during the winter season (most inhabitants use wood for heating, some of them waste and electricity), an influence on the occurrence of blood pressure disorders was confirmed;
- *radiation* (high volume activity of radon from the geological background, radon is considered the carcinogenic element causing lung cancer and respiratory diseases);
- *emissions from rural zone* (agriculture – a smell from the animal husbandry, excrements of animals, liquid manure).

A demographic structure of Kráľovce – Krnišov village population represents a regressive type, lift the biggest age groups in the village between 20–40 and 40–60. Based on the analysis of microclimate, mesoclimate and macroclimate, contamination and illness rate considering the statistic importance, the population group and factors originating from the environment. The occurrence of health problems (Statistically important relations illustrated in Tables I, II, III, IV) could be specified as follow:

- an increased occurrence of the blood pressure disorders in the local population: people using electricity for heating, people using electric appliances at home, women and children, age group over 60, inhabitants suffering from obesity, cardiovascular diseases, occupational diseases and people living mainly on the slopes exposed

Table I
The relation of the diseases occurrence to the sex and age of Kráľovce – Krnišov village population (χ quadrant)

Age/Sex/Affections	Man/age [years]			Woman/age [years]			Child/age [years]		
	20–40	41–60	Over 60	20–40	41–60	Over 60	0–0.5	0.5–12	13–19
Blood pressure disorders	0.76	0.08	0.76	6.17[d]	0.11	10.05[c]	0	0	0
Stress	0.08	0.08	0.76	4.16[e]	0.03	0.50	0.05	0.05	20[c]
Influenza	0.04	0.04	0.33	1.39	0.06	1.08	0.45	0.16	0.01
Eczema	0.04	0.04	0.33	0.67	0.33	2.49	0.19	0.06	0.01
Bronchitis	0	1.37	0.52	0.01	2.27	3.40	0.12	0.09	0.22
Affection from work	0.08	0.08	0.76	1.22	3.26	0.69	0	0	0
Cardiovascular disease	0.83	1.37	0.15	2.67	0.20	1.76	0	0	0
Asthma	0.83	1.37	0.15	0	0	0	0	0	0
Allergies	0	0	0	0	0	0	0.05	0.70	0.57

[c]Relation is at statistically high level of significance (degrees of free1), χ quadrant = 6.63 <
[d]Relation is at statistically medium level of significance (degrees of free1), χ quadrant = 5.02–6.63
[e]Relation is at statistically low level of significance (degrees of free1), χ quadrant = 3.84–5.02

Table II
The relation of the diseases occurrence in Kráľovce – Krnišov village (χ quadrant)

Affections	Blood pressure disorders	Stress	Influenza	Eczema	Bronchitis	Affection from work	Cardiovascular disease	Asthma	Allergies
Blood pressure disorders	–	0.09	1.81	1.66	1.33	8.84[d]	19.44[d]	0.28	0.28
Stress	0.03	–	0.07	0.99	1.65	0.33	0.00	3.99[e]	0.28
Influenza	1.81	0.07	–	0.00	2.46	0.34	1.14	0.22	0.21
Eczema	1.66	0.99	0.00	–	1.56	8.75[d]	2.67	0.18	0.18
Bronchitis	1.33	1.64	2.46	1.57	–	1.09	0.47	8.36[d]	8.36[d]
Affection from work	8.84[d]	0.33	0.34	8.75[d]	1.09	–	13.45[d]	0.12	0.12
Cardiovascular disease	19.44[d]	0.00	1.14	2.68	0.47	13.45[d]	–	0.07	0.07
Asthma	0.28	3.99[e]	0.22	0.18	8.36[d]	0.12	0.07	–	0.01
Allergies	0.28	0.28	0.22	0.18	8.36[d]	0.12	0.07	0.01	–

Table III
The relation of the diseases occurrence to type of dwelling of Kráľovce – Krnišov village pollution (χ quadrant)

Physical, chemical, biological hazards/ affections	Chemicals in work	Manures	Dry toilet	Moisture of house	Electrical applicances	Heating			Mateiral of house	
						Electrical	Waste	Coal	Stone	Brick
Blood pressure disorders	0.09	1.74	4.61[e]	0.30	10.78[c]	12.86[c]	5.07[d]	4.08[e]	0.09	2.25
Stress	4.06[e]	3.13	5.78[d]	4.80[e]	1.36	1.84	2.30	0	0.39	0.27
Influenza	0.00	6.09[d]	4.80[e]	6.09[d]	1.14	3.68	0.03	7.06[c]	0.64	1.54
Eczema	0.04	0.09	0.04	2.12	2.67	0.58	0.47	0.09	0.11	0.39
Bronchitis	1.74	4.30[e]	1.05	2.32	0.65	0.24	0	0.89	0.73	0.90
Affection from work	7.53[c]	4.30[e]	0.05	0.62	13.45[c]	11.11[c]	0.51	0.33	0.04	0.99
Cardiovascular disease	0.06	0.37	0.09	0.01	1.49	5.90[d]	0.03	0.94	0.94	0.08
Asthma	0.20	0.48	1.59	1.66	0.07	0.24	1.76	0.69	5.81d	1.19
Allergies	0.19	0.48	0.51	0.62	0.07	0.24	0.58	0.69	0. 18	1.19

Table IV
The relation of the diseases occurrence to the nutrition and physical work of Kráľovce – Krnišov village population (χ quadrant)

Various/Affections	Finish food	Finish food Smoke	Medicine (ache)	Physicalwork	Sucklening
Blood pressure disorders	4.47[e]	1.17	5.57[d]	0.67	3.16
Stress	0.00	16.63[c]	0.16	2.28	0.99
Influenza	1.08	0.90	0.00	0.34	6.60[d]
Eczema	0.00	0.74	4.54[e]	0.73	0.32
Bronchitis	2.40	0.51	2.45	1.09	0.56
Affection from work	0.96	0.51	2.45	0.03	0.63
Cardiovascular disease	0.13	0.30	3.26	0.47	0.72
Asthma	2.74	0.06	0.62	0.12	0.62
Allergies	0.37	0.06	1.66	0.12	1.66

to the south and on the territory of the "thermal island" effect;

- an increased occurrence of frequent flu in the local population: people living on the slopes exposed, to the age group of children 0–5 months, store-bought meat and house produced eggs consumers;
- an increased occurrence of skin diseases in the local population: the age group of children 0–5 months, adults of the age group 20–40, people living on the places with frequent valley circulation, and people living on the territory with the top climate as a result of the regional air pollution; - an increased occurrence of respiratory diseases in thelocal population: people living on the slopes exposed to the north, on the places with valley climate with important changes of amplitudes and places exposed to the transport emissions, especially with regard to people suffering from allergies, asthma, frequent injuries, radon radiation from the geological background;
- an increased occurrence of cardiovascular diseases in the local population: people living on the slopes exposed to the south, on the territory of "the thermal island "effect, on the territory with the strong changes of amplitudes in the valley climate, people suffering from blood pressure disorders and occupational diseases;
- an increased occurrence of occupational diseases in the local population: people who are in contact with toxic stuff at their workplace and people suffering from blood pressure disorders, skin diseases and cardiovascular diseases.

Conclusions

To determine the relation between climatic conditions and environmental health of the inhabitants of the Královce - Krnišov model village in the Štiavnické Mountains, a new method based on confrontation of potential conditions of selected factors of an environment – climate and real state of the environmental health – was used. Statistical results have shown the relation, which had been theoretically assumed.

After the additional implementation and verification, the model can be considered as a good, quick, cheap and informative method for the assessment of the environmental health in rural settlements.

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L06 INTEGRATION METALLOMICS, PROTEOMICS AND TRANSCRIPTOMICS IN ENVIRONMENTAL ISSUES

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Introduction

Metallomics is one of most recent *-omics* whose importance is associated to the presence of metals or any other heteroelement (e.g. elements different of C, H, N, or O) in biomolecules. These metal-linked molecules play important roles in the cells and by extension in the biological behaviour of the organisms.

Some of these elements are essential for life, marked in green in the Fig. 1, other are non-essential or toxic, marked in red. This is the case of transition elements, such as Fe in Cytochrome P450, a superfamily of enzymes that regulate the metabolism of pollutants, drugs and steroids. As well as Fe in transferrin, that transport and deliver this element. Cobalt is the key-element of B12 vitamin and Ni in urease (the enzyme for urea hydrolysis into carbon hydroxide and ammonia). Other transition elements such as Cu and Zn are responsible for the activity of the superoxide dismutase, which is involved in the elimination of superoxide radical.

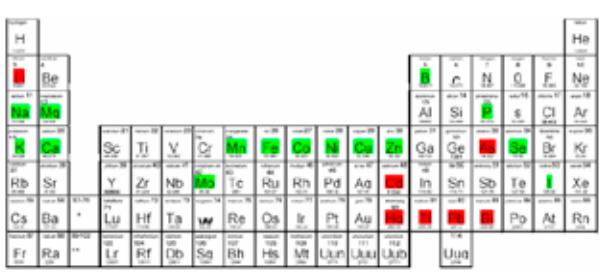

Fig. 1. **Essential (green) and non-essential (red) elements**

Together with elements linked to these proteinous molecules other non-proteinous molecules of high molecular weigh, such as DNA is characterized by the presence of phosphorus, or boron in polysaccharides from vegetal cell walls.

Therefore, the chemistry of a cell and by extension of living tissues and biological fluids can be characterized, not only by its typical genome and proteome, but also by the metallome, the distribution of metals and metalloids among the different biomolecules. The metallome describes, per analogy with genome and proteome, the entirety of metal and metalloid species within a cell or tissue type.[1–4] The scope of metallomics is very broad, focusing on developments of new analytical techniques and instruments, as well as innovative applications focused on environmental, food or health issues.

Instrumental Approaches in Metallomics

Three-dimensional systems should be at least used in Metallomics: *(i)* a separation component by gel electrophoresis or HPLC; *(ii)* an very sensitive elemental monitoring system, for metal or non-metal quantification, mainly ICP-MS; and *(iii)* a component for the structural characterization of the molecules, generally based on mass spectrometry. Therefore, the metal or heteroelement act as tag of the experiment, heteroatom-tagged proteomics[5].

Interest of *–omics* Integration

The benefit that Metallomics produces due to the simplification introduced by the metal tag can be insufficient when an overall appraisal of complex real problems, such as those related to environmental, food or health issues is considered.

If we consider a contamination problem, genetic responses to stress conditions are often regulated at transcriptional level that can be checked by using the microarray technology to generate genome-wide transcriptional profiles. The changes detected by microarrays can be confirmed by RT-PCR (reverse transcription–PCR). In addition, modifications in the proteome can also used as markers of pollution as consequence of protein expression alteration triggered by contamination. However, these changes in proteins profiles can not necessarily reflect alterations in gene expression at the transcript level, but changes from post –transductional modifications.

Therefore, the three *-omics* are complementary and integration among them is advisable. However, the difficulties in integrating data from different *-omics* technologies in non-laboratory strains should not be under-estimated, and the use of non-inbred strains/species induce a variety of confusing factors can complicate interpretation. To avoid these problems in environmental studies we propose the use in parallel of sequenced model species and proved bioindicators with gentic sequence homologous to the model. In the present study we have selected two mouse species: *(i)* a model organism used in many studies in the laboratory, whose genetic sequence can be easily obtaine from database, *Mus musculus*, and *(ii)* an aboriginal species checked as useful sentinel organism in monitoring programs, *Mus spretus*[6].

In the present work a combined application of transcriptomics, proteomics and metallomics approaches has been performed in Doñana Natural Park, one of the most important European biological reserves, in which millions of migrating birds land each year in their way to/from Africa. The couple *M. musculus/M. Spretus* was used for this integration.

Experimental

Sampling Areas

Mice were collected in February 2004 at six sites from Doñana surroundings and the Domingo Rubio Stream, both at Huelva province (Fig. 2.). Animals were captured with live traps and taken alive to the nearest laboratory (Huelva University or Doñana Biological Reserve-CSIC). Their sex and

weight were determined and those of 11–12 g were killed by cervical dislocation and dissected. Individual livers and kidneys were frozen in liquid nitrogen and stored at –80 °C.

Transcript Quantifications

Primer design, RNA preparation, reverse transcription, and absolute quantification by real-time PCR. Briefly, PCR reactions were performed in quadruplicate. No primer dimers were detected. Primers showed optimal (~ 100%) PCR efficiencies in the range of 20 to 2×10^5 pg of total RNA input with high linearity ($r > 0.99$). An absolute calibration curve was constructed with an external standard in the range of 10^2 to 10^9 RNA molecules. The number of mRNA molecules was calculated from the linear regression of the calibration curve ($y = -3.326x + 39.693$; $r = 0.998$).

Microarray-based transcript quantification was performed by using the "Whole Mouse Genome Oligo Microarray Kit" (Agilent), which includes 60-mer oligonucleotide probes representing all known genes and transcripts (~ 41,000) of the model *M. musculus* species. Approximately, 20 µg of total RNA were converted to fluorescently labelled cDNA (with Cy3-dCTP or Cy5-dCTP) following the "Agilent Fluorescent Direct Label Kit" instructions. Hybridization was carried out in Agilent's SureHyb Hybridization Chambers at 65 °C for 17 hours using the Agilent's "Gene Expression Hybridization Kit". The hybridized microarrays were then disassembled and washed at room temperature, as described in the Agilent Microarray Based Gene Expression Analysis protocol. To eliminate dye-bias, dye swap replicates were performed. Microarrays were scanned at 532 and 635 nm using a confocal scanner (Axon 4000B). The ratio of Cy5 to Cy3 was adjusted to 1 varying PMT gain as a global normalization of each array. The images were analysed using GenePix Pro v4.1 software (Axon) and data were subsequently input to Genespring v7.3 software (Agilent) for further analysis.

2-DE Analysis and Protein Identification

Around 50 mg of livers from four male mice/site were pooled and homogenized in 20 mM Tris-HCl, pH 7.6, with 0.5 M sucrose, 0.15 M KCl, 20 mM DTT (Dithiothreitol), 1 mM PMSF (phenylmethanesulfonyl fluoride), and protease inhibitors, at a ratio of 3 ml g^{-1}. Cell debris was cleared by centrifugation, and the supernatant treated with benzonase and ultracentrifuged. Protein extract (115 µg) was incubated 30 min in 450 µl rehydration buffer (7M urea, 2% CHAPS, 20 mM DTT, 0.5% Pharmalyte 3–10, bromophenol blue traces), spun and loaded on 24 cm (pH 4–7) Amersham Immobiline Dry-Strips®. After 6 h passive and 6 h active (50 V) rehydration in a BioRad Protean IEF cell (20 °C, 50 mA strip^{-1}), the voltage was raised until obtain ootimum separation. After freezing at –80 °C, the strips were soaked 20 min in equilibration mix (50 mM Tris-HCl, pH 8.8, 6M urea, 30% glycerol, 2% SDS, bromophenol blue traces) with 65 mM DTT, drained and again soaked 20 min in this mix with 25 mM iodoacetamide. SDS-PAGE was done in 12.5% gels using the BioRad Protean® Plus Dodeca cell (20 °C) at 2.5 W gel^{-1}, 10 min, and 10 W gel^{-1} until separation was finished. Gels were silver-stained following a standard protocol compatible with MS analysis. Analytical quality chemicals and Milli-Q water (Millipore®) were used throughout.

Gel images of three replicates/sample were obtained with a BioRad GS-800 densitometer. Spot volumes were quantitated using the PDQuest software (v7.1, BioRad). Initially, only spots exhibiting in "Santa Olalla" lagoon (SOL) an over/underexpression ratio of at least threefold with respect to any other sampling site were considered. One-way analysis of variance followed by the Student–Newman–Keuls post-test was then used for a definitive selection of the spots showing altered expression patterns between the different animal groups. Differentially expressed spots were manually excised, reduced (10 mM DTT), alkylated (55 mM iodoacetamide), digested overnight at 30 °C with trypsin (Promega) and the peptides extracted with ACN/TFA (Acetonitrile/Trifluoroacetic acid). Aliquots of 0.5 µl were analyzed by MALDI-TOF-PMF (Matrix Assisted Laser Desorption- Time of Flight-Peptide Mass Fingerprint) in a Voyager DE-PRO instrument (Applied Biosystems) in reflectron mode. PMF data were contrasted against mammalian sequences included at Swiss-Prot (EBI, Heidelberg, Germany) and nonredundant NCBI (Bethesda, MD, USA) databases using ProteinProspector (California University, San Francisco, CA, USA) and MASCOT (Matrix Science, London, UK) softwares.

Analysis of Extracts by SEC Coupled with ICP-MS

Extracts were twofold diluted with the mobile phase and centrifuged at (11,000 rpm) $15.5572 \times 1g$ for 1 h at 4 °C, and latterly filtered through Iso-Disc poly(vinylidene difluoride) filters (25-mm diameter, 0.2-µm pore size) to avoid column overloading or clogging. Elemental fractionation profiles were obtained by size exclusion chromatography (SEC) coupled to ICP-MS as detector. Two columns were used in the experiment: Hiload 26/60 Superdex 30 Prep column for a separation range below 10 kDa (low molecular mass, LMM) and a Superdex 75 Prep column for a separation range of 3–70 kDa (high molecular mass, HMM), both from Amersham Biosciences (Uppsala, Sweden). These columns were calibrated using standards of known molecular mass, such as bovine serum albumin (67 kDa), metallothionein I (7 kDa), gastrin rat I (2,126 Da) and Gly6 (360 Da) for LMW column, and bovine serum albumin (67 kDa), chymotrypsinogen A (25 kDa), ribonuclease A (13.7 kDa) and metallothionein I (7 kDa) for HMW column. The void retention time was estimated with bovine serum albumin (67 kDa) and blue dextran (2,000 kDa), for LMW and HMW, respectively.

Results

The three *-omics* approaches (transcriptomics, proteomics and metallomics) have been applied to Doñana Natural Park and the surrounding areas. Doñana is an impor-

Fig. 2. **Sampling area**

tant ecological area, which covers 543 km^2 with a great variety of ecosystems and shelters wildlife including thousands of European and African migratory birds, fallow deer, Spanish red deer, wild boar, European badger, Egyptian mongoose, and endangered species such as the Spanish Imperial Eagle and Iberian Lynx. This non-contaminated area was used as negative reference (SOL) in comparison to the neighbouring "Domingo Rubio" stream (DR1 to DR6) and the positive references (PS and ARZ). These areas are contaminated by mining, agricultural and industrial effluents (DR1 to DR6 and PS) and by pesticides and fertilizers (ARZ) (Fig. 2.).

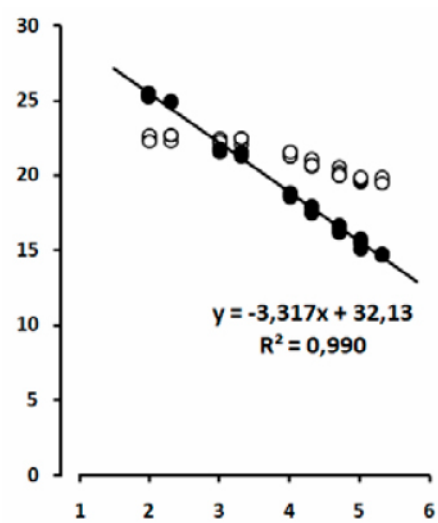

Fig. 3. **Efficiency curves in genetic correlation between *M. musculus* and *M. spretus* for Gsta3 transcript. ● specific primers, ○ non-specific primers. X-axis, log total mNRA (pg); Y-axis, threshold cycle (Ct)**

The mouse *Mus spretus* is an aboriginal species that has been commonly used for environmental assessment of this area (x) by means of classical biomarkers, but new tools based on recent –omics, such as transcriptomimcs and proteomics constitute a promising alternative. However, the molecular biology methodologies present problems by the fact of poor inclusion of typical bioindicators in gene/protein sequences database. For this reason we use species close to model organisms that are well covered in public databases. This is the case of *M. musculus*, which is studied comparatelly with *M. spretus*. These comparisons are only possible if genetic homology between both species is proved.

Genetic Homology Between *M. musculus* and *M. spretus*

A crucial start-point in quantitative RT-PCR is primer design. For absolute transcript quantification it is necessarily to design primers that amplify the targets and the calibrator with optimal (100 %) PCR efficiencies. This fact requires a great genetic homology between target and calibrant species. Primers for RT-PCR quantification of *M. spretus* Cyp and Gst mRNAs were designed based on known gene sequences from *M. musculus*. Remarkably, these primers, when amplified in *M. spretus*, gave single products exhibiting in most cases 100% nucleotide sequence identity. Therefore, most designed primers were exactly complementary to the desired M. spretus templates and amplified them with 100% efficiency. In few cases, however, primers should be redesigned based on nucleotide sequences of PCR fragments from *M. spretus*. Fig. 3 ref.[8].

Therefore, genetic homology between model and bioindicator species was clearly proved.

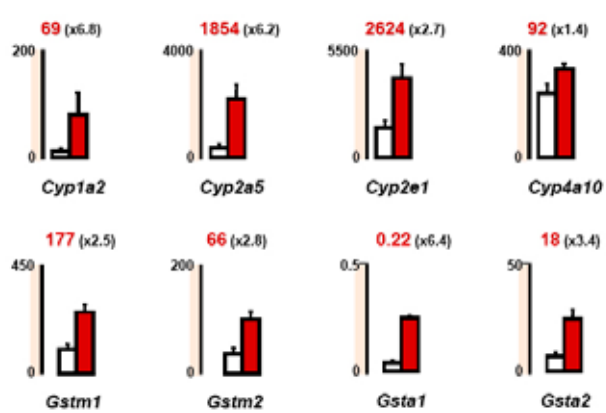

Fig. 4. **Sampling area-associated differences in *M. spretus* hepatic mRNA levels. Y-axis, nRNA molecules/pg total RNA. White bar, SOL non-polluted area; red bar, polluted area**

Mus Spretus Transcript Expression Signatures in Contaminaed Areas

We have used the absolute measurement of mRNA levels from selected key genes (CYPs and GSTs) to biomonitoring the exposure and biological effects of pollutants on free-living nonmodel *M. spretus*. For this purpose the mRNA molecules of genes coding for different cytochrome P450 and glutathione transferases were quantified in mice dwelling at both the non-contaminated point SOL and contaminated area PS.

As an example, Fig. 4. shows the concomitant up-regulation of some *Cyp* transcripts in *M. spretus* PS population, as compared to that at SOL in the Doñana Biological Reserve. The possibilities of transcription quantification to assess the level of contamination are clearly demonstrated. In addition, the absolute Cyp transcript expression signature is depending on the type of contaminant (Fig. 5.), which can use to identify the nature of contamination under consideration.

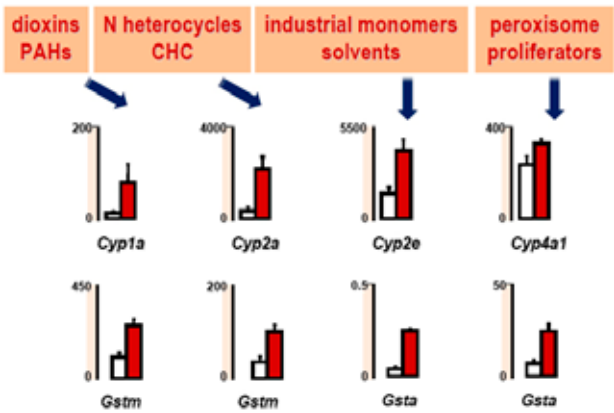

Fig. 5. **Transcripts expression signature related to the type of contaminants. Y-axis, nRNA molecules/pg total RNA**

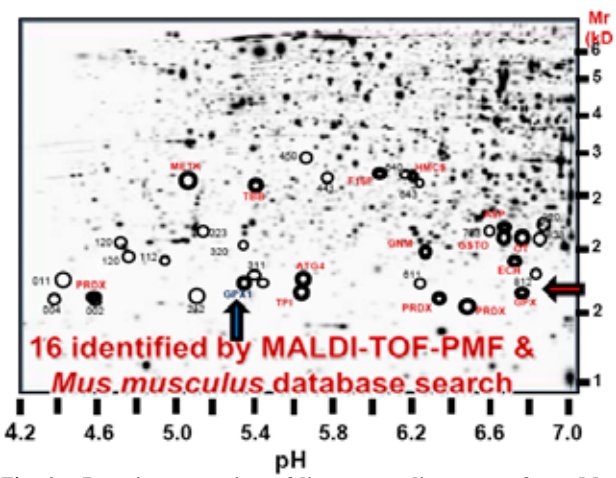

Fig. 6. **Protein expression of liver cytosolic extract from *Mus spretus***

Proteomics Study of *Mus spretus*

The homology between *M. musculus* and *M. spretus*, at DNA sequence level allows the proteomics evaluation of protein expression differences caused by contamination episodes in *Mus spretus*, but usinf the gnetinc sequence of *Mus musculus* in protein identificacion by MALDI-TOF-PMF. A comparative study at proteomic level of cytosolic fractions of liver from *M. spretus* sampled in Doñana and Domingo Rubio stream were performed analysing the extracts by 2-DE and searching the protein expression differences. Over 2500 spots were resolved in the pH range 4–7 and 14–70 kDa M_r. Image analysis of the gels yielded 36 spots with significantly altered expression. Of them, 16 proteins were identified by MALDI-TOF-PMF and heterologous search against *Mus musculus* databases. When this approach is applied to contaminated and non-contaminated points a clear diference in spots intensities corresponding to differentially expressed protreins was observed, which can be used for the environmental pollution assessment of proteomics.

Metallomics Approximation to the Mouse *Mus musculus*

In this study the presence of unknown metallobiomolecules in *M. musculus* was studied for the first time. Firstable, a general evaluation of the presence of total concentration of metal in different organs of the mouse (lung, liver, spleen, kidney, brain, testicle, heart and muscle) was performed.The experiments were carried out on inbred *M. musculus* specimens. An you can see the elements considered. Fe is the element most abundant in the different organs with an averaged concentration of 7,500 $\mu g\,dm^{-3}$ in thecytosolic extracts. Other elements are Zn (about 2,300 $\mu g\,dm^{-3}$), Cu (550 $\mu g\,dm^{-3}$), Ni (165 $\mu g\,dm^{-3}$), Se (145 $\mu g\,dm^{-3}$), and a toxic element such as Pb (26 $\mu g\,dm^{-3}$). These elements are differentially distributed in the organs, for example, Cu and Zn are mainly present in the liver, but Pb in the muscle. An similar comments can be addressed to the other elements.

Metal-biomolecules profiles was obtained with ICP-MS as metal tracer, using couplings with size exclusion chroma-

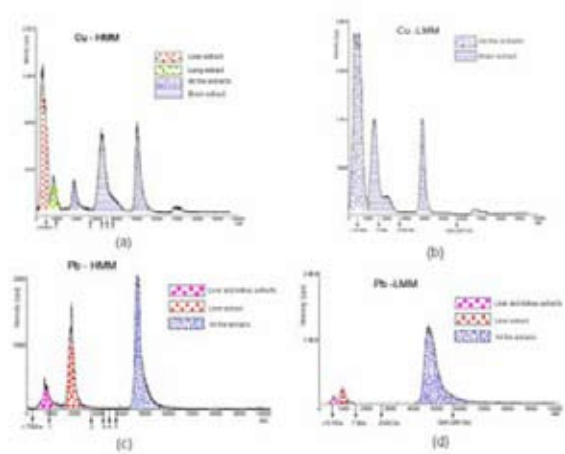

Fig. 7. **SEC-ICP-MS metal profiles in *Mus musculus* corresponding to Cu and Pb. HMM size exclusion column for 3,000 to 70,000 Da; LMM, size exclusion column for 300 to 10,000 Da**

tography to characterize metalbiomolecules molecular mass profile, and subsequently in series, on the fractions isolated in the previous separation, reverse phase chromatography for a further purification of the extracts and biomolecule isolation. Two SEC columns were used to discriminate molecules with molecular mass range under 10,000 (360 to 10,000 Da) and between 3,000 and 70,000 Da. The results for Cu and Pb, this latter as example of toxic are shown as examples in Fig. 7.

The results obtained for Cu and Pb can be summarized in the following items:

- All extracts showed peaks in the range of 300 and 2,000 Da whatever metal or organ considered.
- A peak was observed in the void volume of the LMM column ($M_r < 10$ kDa) for all profiles, although they showed low intensities except for Cu that was studied in more detail.
- A Cu-containing fraction (7–10 kDa) was found in the brain which was absent in all other M. musculus organs. Other elements, e.g. Pb, do not have peaks equivalent in that range of masses. Therefore, further studies involving subsequent purification by another chromatography technique and isolation of this Cu-bound biomolecule for MS identification is being considered.
- The Cu and Pb fractions resolved in the HMM column were mainly associated with molecules with molecular masses from 25 to 67 kDa, which are present in all the organs for Cu and only in liver for Pb.
- A chromatographic peak associated to Cu was detected in the molecular mass range 67 to 70 kDa for the lung extract, and in liver and kidneys for Pb.
- A Cu-peak can be observed in liver extract at the void volume of the HMM column.

In Fig. 8. is shown the chromatograms correponding to the cytosolic extracts. The main fraction is associated to liver and is eluted in the void volume of HMM column. Additional experiments with reverse phase chromatography of this isolated fraction and ICP-MS detection reveal that tow peaks can result from additional purification of this fraction.

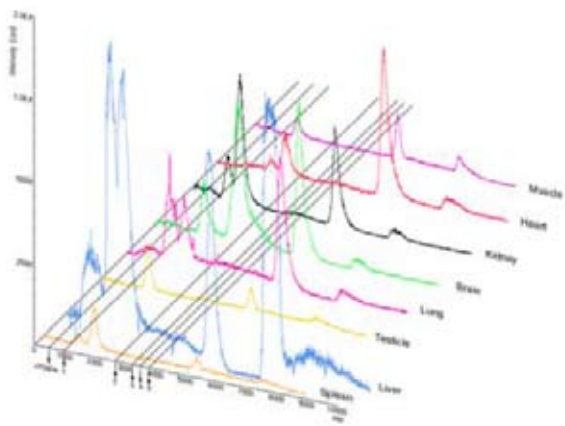

Fig. 8. **Chromatographic profiles with HMM column (3,000 to 70,000 Da)**

Integration of Metallomics with Proteomics and Transcriptomics

The complementary character of the different approaches presented in relation to *Mus musculus*/*Mus spretus* and the environmental assessment considered prevously claims for the integration of these three -*omics*.

The availability of the complete sequence of an increasing number of genomes allows the developments in proteomics, which can be achieved by electrospray and MALDI-TOF mass spectrometry. However, many proteins can be sensitively detected by ICP-MS due to the presence of ICP-ionizable elements (heteroelements). Therefore the three -omics are complementary, and in this context the combination of validated metallomic data with the transcriptome and proteome knowledge of a cell is one of the largest challenges for future research in this topic.

A crucial question is that better established -*omics* (transcriptomics and proteomics) support their studies on model organisms whose gene/protein sequence are well collected in databases. On the contrary, bioindicators currently used in environmental studies are non-model organisms absent from public databases. The design of primers that allows the confirmation of homology in gene expression at the transcript level between bioindicators used in environmental studies and sequenced model specimens is a very good tool to perform environmental metallomics and proteomics.

This new triple-omics approach (MEPROTRANS-*triple*-OMICS) is being applied to the *M. musculus*-*M. spretus* couple in Doñana Natural Park for environmental assessment, following the steps listed below:

- Extraction of RNA for transcriptomic and of proteins for proteomic and metallomic analyses.
- Quantification of changes at the transcriptome level by means of commercially available microarrays and further validation of selected microarray results by absolute quantification using real-time RT-PCR.
- Quantification of changes at the proteome level by 2-DE analysis and subsequent protein identification by MALDI-TOF-PMF.
- Integration of transcriptomic and proteomic results to distinguish transcriptional from post-transcriptional changes.
- Isolation of heteroatom-tagged molecules by size exclusion chromatography fractionation of tissue extracts guided by ICP-MS (First Dimension) and further purification by reverse phase and/or ion exchange chromatography with ICP-MS on the previous selected fractions (Second Dimension). This metallomic scheme will be assisted by 2-DE analysis to reveal the complexity of the successive sub-proteomes. The purification steps involving metallomics and proteomics will be repeated until the final isolation of target metal-biomolecules for identification by tandem mass spectrometry.

- Validation of metallomic results by Western hybridization and/or enzymatic assays, and by absolute quantification of transcripts coding the identified metalloproteins.
- Integrative multidisplinary decision on the optimal assay (high sensitivity, low cost and easy to perform) to be recommend for routine assessment of differentially expressed metalloproteins as novel biomarker in ecotoxicological studies.

Conclusions

The study of complex systems related with living organisms, such as the environment or health, in which many variables are involved, requires multidisciplinary tools for a comprehensive assessment of these issues.

Several –*omics*, such as transcriptomics, proteomics and metallomics, offer a valuable alternative in environmental studies since they provide massive information about biomolecules in cells and organisms that help to overcome these problems. An overall evaluation of changes that contaminants induce in cells is only possible by integration of -*omics*:

- Transcripts induced by pollutants (transcriptomics) encode proteins with altered expression profiles, which undergo post-traductional modifications (proteomics).
- Many proteins related to enviromental issues are bound to metals (i.e. CYPs, SODs, GPXs, metallothioneins) that make advisable the use of metal-tagged techniques (metallomics) as preliminary step to simplify proteomic and transcriptomic approaches.

Proteomics can guide metallomics by checking the presence of molecules in extracts obtained with the chromatography-ICPMS couplings:

- This assists metal biomolecules isolation for mass spectrometry identification.
- 2D analysis provide an image of the number of molecules in the extract and their molecular mass

This triple -*omic* approach (MEPROTRAN-*triple*-OMICS) is a very useful and comprehensive alternative in the study of environmental issues and the diagnosis of contamination problems. In addition, MEPROTRANS-*triple*-OMICS can assist in the validation of traditional biomarkers and a more simple cheap and fast assessment of environmental issues.

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L07 HEAVY METALS IN SOLID IMMISIONS IN THE VICINITY OF IRON ORE MINING AND PROCESSING PLANT IN NIŽNÁ SLANÁ

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Introduction

The content of hazardous substances in atmospheric deposition significantly contributes to the pollution of environment. A lot of studies deals with the research of atmospheric deposition, mostly using methods monitoring wet deposition, wet and dry deposition (bulk deposition), but also methods using fog and low cloud sampling[1]. The solid emissions from the technologies of ores and industrial minerals processing by their specific composition influence constitution of atmospheric deposition, especially in the areas of processing plants. The main emission source in the area of Nižná Slaná is iron-ore mining and processing plant. The plant exploits the siderite ore. The run-off-mine ore is through several technological centres processed into the blast furnace pellets as a final product of the plant. The contribution deals with the evaluation of results obtained from monitoring of atmospheric deposition in the form of dust fallout (modified method bulk deposition). The research was carried out by the Institute of Geotechnics of the SAS in the area of the Siderite, Ltd. from 2001 to 2007, predominantly from viewpoint of heavy metals deposition (Fe, Mn, Zn, Pb, Cu, Cr, Cd, and As).

Characteristics of the Plant

The plant is situated in the Slaná river valley in the Slovak Republic. The valley has an orientation of north-south and northwest-southeast, respectively. The wind circs are influencing by an orography of given territory. Distribution of wind directions and the occurrence of calm in the near-by Rožňava town are shown in Table I.

Exploited deposit is located in the Revúca upland Mts., the Dobšiná foothill belt Mts. of the Slovak Ore Mts. Utility mineral is siderite. The average content of iron and manganese in ore is 33.5 % and 2.8 %, respectively. Manganese is bonded isomorfically in the siderite lattice. There are also unfavourable elements (As, S, Pb, Zn) that occur as, sulphides, sulphates, sulphosalts and oxides. The most significant unacceptable impurity is arsenic that is present in the form of arsenopyrite. The average content of As in the run-off-mine ore is approximately of 0.01–0.1 %. Ore processing consists of crushing, magnetizing roasting, wet milling, magnetic separation and pelletizing. Primarily, thermal technologies, i.e. pelletizing and magnetizing roasting are responsible for the amount of dust outlet. Flue gases are exhausted to the environmental air through 120-meter high chimney after several stages of dedusting. Table II presents the average concentrations of the selected elements in the dust outlet from pelletizing plant and rotary furnaces. The emissions of solid pollutants from 1998 to 2007 are shown in the Table III.

Material and Methods

The samples of dust fallout were taken in the 30 days (± 3 days) intervals from the seventeen sampling stations. The cylindrical plastic sedimentation containers (inside diameter – 12.5 cm), put in two support stands in the height of 2.5 to 3 m, were used for the sampling. The containers were filled with 250 ml of pure water with addition of isopropanol. After sampling, the content of containers was quantitatively located to evaporating dishes and evaporated. The organic mass was removed by annealing of dry matter at 450 °C. The chosen temperature prevented carbonate degradation and in such way enables to avoid the misinterpretation of dust fallout gravimetry results. The samples were gravimetrically evaluated before and after annealing, in the mass units recalculated for the area and the respective time period. Inorganic portion determined by annealing from twelve month period was cumulated to the one sample and after mineralization it was analysed using AAS (SpectrAA – 30 VARIAN). On the basis of these chemical analyses and mass yield of the dust fallout, the average annual depositions by observed heavy metals were calculated for each of the seventeen sampling points. The localization of sampling stations is illustrated in Fig. 1.

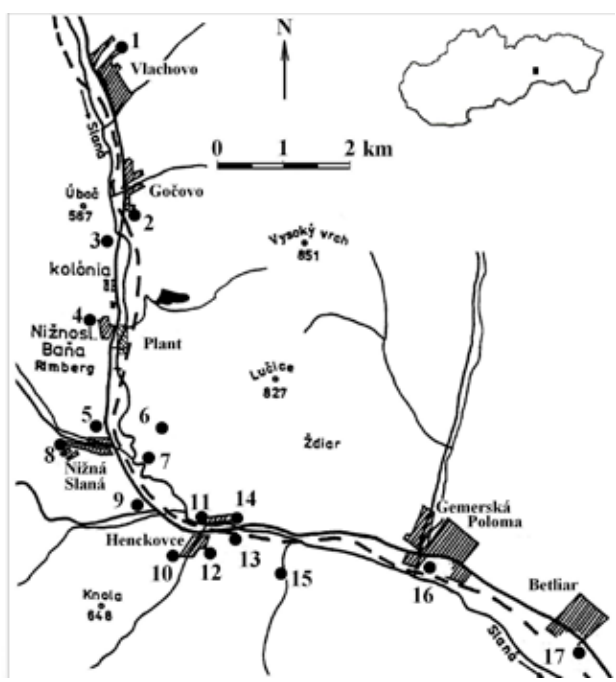

Fig. 1. **Sampling locations in the area of Nižná Slaná**

Results and Discussion

The average year values of total deposition for whole monitored period determined for individual sampling points

Table I
Percentage of the wind directions and calm in the Rožňava town [%]

N	N-E	E	S-E	S	S-W	W	N-W	Calm
38	8	6	2	25	2	3	6	10

Table II
The content of the selected elements in the solid dust outlets from thermal technologies

Technologies	Fe	Mn	Zn	Pb	Cu	Cr	Cd	As
	[%]		[ppm]					
Pelletizing	30.4	3.48	94	32.6	99	39	25	636
Rotary furnaces	27.8	2.12	200	127	170	63	24	176

Table III
The emissions of solid pollutants from the plant (1998–2007) [t year^{-1}]

1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
156.4	116.3	116.9	86.7	32.9	63.3	63.8	121.5	155.8	148.4

Table IV
The average deposition of selected heavy metals and total deposition in the area of Nižná Slaná (2001–2007)

Parameter	Total dep.	Fe	Mn	Zn	Pb	Cu	Cr	Cd	As
	[mg m^{-2} year^{-1}]								
Minimum	8,621.0	1,221.0	60.9	9.54	0.35	1.67	1.09	0.033	1.49
Maximum	32,537.0	9,636.0	631.3	63.12	2.81	5.73	4.36	0.078	28.84
Average (n = 17)	18,155.0	4,543.0	272.7	23.34	0.93	3.38	2.58	0.050	11.07
Median	17,173.0	4,423.0	265.0	17.68	0.68	3.27	2.59	0.048	8.77
SD	6,996.0	2,288.0	160.6	16.59	0.65	1.05	1.05	0.012	7.50

were in the interval 8.6–32.5 g m^{-2} y^{-1}. In most of sampling points a significant increase of solid pollutant emissions in 2005 till 2007 comparing with previous years was not detected (total dust fallout). On the other hand, the significant increases of Fe, Mn and As deposition values were recorded using dust fallout analysis in the most of sampling stations. The samples of dust fallout were also subjected to X-ray diffraction (XRD) analysis. Thus, minerals such as quartz, chlorite, siderite, ankerite were detected. These minerals come from raw ore handling but also as a result of aeolation from surrounding environment. On the other side hand there are minerals, namely hematite, maghemite, magnetite and wüstite coming from the thermal technologies of the plant[2].

Table IV presents average values of deposition of selected heavy metals and basic statistic parameters for the whole monitored period and all sampling points. Qualitative composition of dust fallout unlike of its quantity significantly contributed to the ecological load of the individual sampling points that was caused by plant activities. Between various sampling points high differences in the deposition were determined mainly for Fe, Mn and As. There were not so high differences in the deposition with others elements. The most ecological load was situated in the south of the plant, in the central part of the valley. The statistical dependence between emissions of solid pollutants and deposition of observed metals (annual median) in the individual years by correlation analysis was evaluated. Relatively high positive values of correlation coefficient were calculated for manganese, iron and arsenic, namely 0.794, 0.764 and 0.749, respectively. Correlation coefficients for other elements were relatively low. The development of selected metal deposition (median) and the emissions of solid pollutants for the whole monitoring period is showed in Fig. 2.

In the Slovak Republic there are not determined allowable limits for heavy metals deposition from dust fallout. That is the reason why relative comparison of heavy metal depositions for different localities is used for assessment of immission load.

In the Nižná Slaná locality, Fe deposition achieves high values because of solid pollutants emissions consist mainly of iron minerals. Fe depositions monitored in the individual stations were in the range from 1.221 to 9.636 mg m^{-2} y^{-1}. In Košice town with metallurgy industry, the values varied from 232 to 7.568 mg m^{-2} y^{-1}(ref.[3]). In the Czech Republic

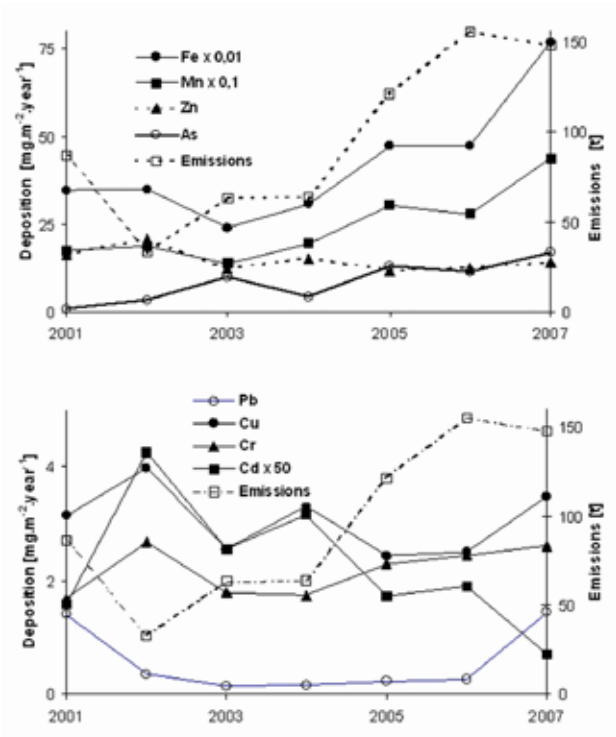

Fig. 2. **Development of selected metal deposition (median) and the emissions of solid pollutants (2001–2007)**

in 2005 (measured for subsystems of basic and contaminated areas), ten times lower Fe deposition was determined in comparison with area of Nižná Slaná (69–1,002 mg m^{-2} y^{-1}) (ref.[4]).

The highest contents of manganese in dust fallout were detected in the area of Nižná Slaná and Jelšava with maximum deposition values of Mn 631.3 and 406.4 mg m^{-2} y^{-1}, respectively[3]. Average values exceeded 200 mg m^{-2} y^{-1}. In past, the most manganese loaded territory was the locality Dolný Kubín that is located nearby iron-alloy factories Istebné, where the maximum deposition Mn value from dust fallout was 668 mg m^{-2} y^{-1}(ref.[5]). In other parts of Slovakia, average deposition of Mn from dust fallout were 3.6 and 34.4 mg m^{-2} y^{-1} in the Slovak Paradise and Košice, respectively[3]. In the case of Nižná Slaná and Jelšava, the main manganese sources were iron-ore mining factories, magnesite factories and raw materials (siderite, magnesite) treated in these factories. In the Czech Republic in the monitored territories the average deposition of Mn was 13.6 mg m^{-2} y^{-1} and the maximum deposition was 68.3 mg m^{-2} per year[4].

The deposition values of Zn in the Nižná Slaná area were in the interval 9.5–63.1 mg m^{-2} y^{-1} with the average value of 23.3 mg m^{-2} y^{-1}. In general, these values are not very high. In the Czech Republic, the average atmospheric depositions of Zn were 50.6 and 54.3 mg m^{-2} y^{-1} and the maximum was 206 mg m^{-2} y^{-1}(ref.[4]). In the agricultural area Austria and England, the measured values of zinc deposition were in range from 21.4 to 48.9 mg m^{-2} y^{-1} respectively 22.1 to 35.6 mg m^{-2} y^{-1}(refs.[6,7]).

The deposition of Pb was relatively low (0.11–2.44 mg m^{-2} y^{-1}) in the locality of Nižná Slaná. The maximum content of Pb (21.89 mg m^{-2} y^{-1}) from dust fallout was measured in Krompachy, while the main source of solid pollutions was cooper smeltery[3]. In past, in Slovakia, there was determined the maximum content of Pb in dust fallout (380 mg m^{-2} y^{-1}) in the locality of Prievidza, that was caused by steam brown coal combustion[5]. The deposition of Pb was 5.4 to 13.9 mg m^{-2} y^{-1} in England and Wales[6]. In Austria, the measured values were 1.90–5.44 mg m^{-2} y^{-1}(ref.[7]).

The deposition of Cu achieved low values in Nižná Slaná (1.74-5.68 mg m^{-2} y^{-1}). High copper deposition (128.26 mg m^{-2} y^{-1}) was measured in Krompachy[3]. The values in Austria and England were in range from 6.24 up to 14.70 respectively 22.1 to 35.6 mg m^{-2} y^{-1}(refs.[6,7]) .

The average depositions of chromium in Nižná Slaná were in the interval from 1.19 to 4.77 mg m^{-2} y^{-1}. The average depositions of chromium from dust fallout in the Slovak Paradise and Jelšava were 0.64 and 10.52 mg m^{-2} y^{-1}, respectively. The maximum measured value was detected in Košice (14.62 mg m^{-2} y^{-1})(ref.[3]). In the Czech Republic, atmospheric deposition monitoring revealed values in the range from 0.21 to 2.81 mg m^{-2} y^{-1} and in Austria in the interval from 0.72 to 1.95 mg m^{-2} y^{-1}(refs.[4,7]). In England and Wales, the Cr values were in range from 0.75 to 2 mg m^{-2} y^{-1}(ref.[6]).

The deposition values of cadmium in the Nižná Slaná were in the range from 0.033 to 0.084 mg m^{-2} y^{-1}. This is relatively low value. In Košice and Jelšava, the measured average values were 0.678 and 0.742 mg m^{-2} y^{-1}, respectively[3]. In the Czech Republic, the atmospheric deposition monitoring detected values in the range from 0.025 to 1.230 mg m^{-2} y^{-1} and n Austria in the interval 0.146–0.325 mg m^{-2} y^{-1}(refs.[4,7]). The deposition of Cd was 0.19 to 0.61 mg m^{-2} y^{-1} in England and Wales[6].

Relatively high values of arsenic deposition were registered in the locality of Nižná Slaná. The average values in the monitored areas were in the interval of 1.28–29.28 mg m^{-2} y^{-1}. The source of arsenic is arsenopyrite that is present in siderite batch. The maximum arsenic value was detected from sampling station no.5 that is close to the factory. This value is two fold times higher that the maximum value from Krompachy, where the measured value was 14.588 mg m^{-2} y^{-1}(ref.[3]). In the relative pure area of Slovak Paradise, the recorded values of arsenic deposition were from 0.044 to 0.155 mg m^{-2} y^{-1}(ref.[3]). The atmospheric deposition monitoring in the Czech Republic registered values from 0.21 to 2.81 mg m^{-2} y^{-1}(ref.[4]). In past, in the Slovak Republic, maximum content of As in dust fallout was 35 mg m^{-2} y^{-1} in the locality of Prievidza (effect of steam brown coal combustion with high concentration of As)[5]. The average values for atmospheric deposition in the Czech Republic were 0.50–0.67 mg m^{-2} y^{-1} and maximum arsenic value was 2.11 mg m^{-2} y^{-1} in 2005(ref.[4]). In England and Wales, the As values were in range from 0.31 to 1 mg m^{-2} y^{-1}(ref.[6]).

Conclusions

The detailed analysis of dust fallout from the locality of Siderite, Ltd. factory revealed the extent of ecological load. In comparison with others localities, this area is the most influenced with heavy metals (iron, manganese and arsenic). This fact reflects the composition of siderite and applied technological treatments of siderite ore in the factory. Although relatively small territory was monitored, the significant differences are detected. There are many different factors, for example: meteorological, orographic, emissive, binding and particle sedimentation mechanisms with linkage to impurities and also others factors. Although the method of measuring of dust fallout is laborious with some mistakes, the relative evaluation of individual localities imission load provides applicable results.

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L08 COMPARISON OF ENERGY DISPERSIVE X-RAY FLUORESCENCE SPECTROMETRY, INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROMETRY AND LASER ABLATION WITH PLASMA SPECTROMETRY IN THE ELEMENTAL ANALYSIS OF SOILS

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Introduction

Agricultural soils represent complex multiphase multicomponent material which requires adequate methods of chemical analysis. Content of elements represents important parametre which relates to nutrition of plants on one side and a contamination with toxic elements on the other side. For intake of elements by plants only selected elemental forms (species) are bioavailable. Speciation or fractionation based on selective leaching of particular species followed by atomic absorption spectrometry or inductively coupled plasma optical/mass spectrometry (ICP-OES, ICP-MS) analyses of resulting solutions are employed for this purpose. Nevertheless, total elemental contents, which comprise also elemental forms that are insoluble in leaching media or by the action of plants, might be interesting for overall characterisation of a particular soil. In this case, total sample decomposition procedures based on the action of a mixture of mineral acids or a sample fusion with a suitable melt are applied to dissolve resistant minerals and desintegrate possibly present silicate lattice.

However, some methods for direct analysis of solids are more advantageous because of elimination of possible analytes losses and minimization of risk of contamination. For this purpose, X-ray fluorescence (XRF) spectrometry[1] is frequently and routinely used. Laser-assised plasma spectrometry techniques, such as laser induced breakdown spectroscopy[2], and ICP-OES or ICP-MS in connection with laser ablation (LA) seem to be promising tool for soil analysis.[3–7]

The aim of this work consists in establishing new methods of soil elemental analysis using LA-ICP-OES/MS. Artificially intentionally contaminated archive soil samples were employed. Toxic metals Cr, Ni, Cu, Zn and Pb were considered in the method developement. For the method validation, these soil samples were subjected to ICP-OES analysis of solutions obtained by total decomposition using mineral acids. Seleted soil standard reference materials were also analyzed. Independent results acquired by XRF analysis of soil sample pellets were used for confirmation of accuracy of solution analysis by ICP-OES.

Besides the total elemental content determination, selected soils were characterized by means of the five-step extraction procedure to asses elemental fractions bound to particular soil phases. This information might be important for explanation of possible matrix effects associated with processes of LA.

A selection and preparation of a suitable binder represents the indispensable step in the development of ablation – based methods. In our work we investigated a silica sol-gel matter preparation and its mixing with soil samples. The binder was then successfully applied in LA-ICP-OES/MS methods. The accuracy of the developed methods was confirmed by analysis of certified reference materials of soils (GBW07405 – 07).

Experimental

Soil Samples

Experimentally contaminated agricultural soils representing various soil types belong to archived materials of the Central Institute for Supervising and Testing in Agriculture (UKZUZ), Brno, Czech Republic.

Studied trace elements Cr, Ni, Cu, Zn, Pb occur in the concentration ranges as shown in Table I.

The soil samples were ground in a ball mill and dried to constant weight. The particle size of ground samples was measured by laser diffractometry and the particle diameter did not exceed 30 μm.

Table I
Concentration range of tested soils and RSD values for PN-ICP-OES

Element	C range [mg kg^{-1}]	RSD [%]
Cr	21–590	3.7–1.7
Ni	3.5–262	14–0.9
Cu	11–242	2.5–3.2
Zn	51–1355	2.7–0.5
Pb	22–1015	2.7–1.6

The solution analysis by ICP with pneumatic nebulization, (PN-ICP-OES) was carried out after the sample decomposition with a mixture of HF and $HClO_4$(ref.[5]) The soil-wax pellets for XRF analysis were prepared as a mixture of 4 g of a soil powder and 0.9 g of wax. The mixture was homogenized in a ball mill and then pressed by a hydraulic press to a pellet with a diameter of 32 mm. Sequential extraction was performed using five reagents with a different strength (Table II)[8,9]. The sol-gel method, allowing homogenous dispersion of internal standard (Sc) and analytes in calibration pellets, was applied for analysis of tested soils by LA-ICP-MS[10].

Table II
Sequential extraction procedure

Step	Metal phase	Reagent
1	Exchangeable	1 M NaOAc (pH = 8.2)
2	Carbonate	1 M NaOAc (pH = 5)
3	Mn-Fe oxides	0.04 M $NH_2OH \cdot HCl$ in 25 % HOAc
4	Organic	0.02 M HNO_3, 30% H_2O_2(pH = 2), 3.2 M NH_4OAc in 20% HNO_3
5	Residual	conc. HF, $HClO_4$, HCl

S o l - G e l T e c h n i q u e

Sol-gel is an amorphous solid prepared via sol-gel processing, in which a colloidal suspension (sol) is first formed by mixing a silicon (e.g. tetraethoxysilane-TEOS) alkoxide precursor, water, a co-solvent and an acid or base catalyst. Alkoxyl groups are removed by acid- or base-catalysed hydrolysis reactions (Eq. (1)), and networks of O – Si – O linkages (gel) are formed in subsequent condensation reactions, which can produce either water or alcohol as shown in equations (2) and (3), involving hydroxyl groups[11]. Internal standard is integrated with the sample during gelation process.

Hydrolysis:

$$Si(OR)_4 + H_2O \rightarrow Si(OR)_3OH + ROH \quad (1)$$

Condensation:

$$X_3SiOH + HOSiX'_3 \rightarrow X_3Si-O-SiX'_3 + H_2O \quad (2)$$

$$X_3SiOR + HOSiX'_3 \rightarrow X_3Si-O-SiX'_3 + ROH \quad (3)$$

An archive soil sample with the lowest content of Cr, Ni, Cu, Zn and Pb spiked with solutions of elements of interest was used for the preparation of calibration samples. Twenty-two soil samples and three certified reference material of soils (GBW07405 – 07) were analyzed by developed LA-ICP-MS method and the obtained contents were compared with results of independent methods (XRF, ICP-OES).

The real soil samples were treated by the following procedure: 1.2 g of soil was weighed into a 50 ml beaker and 3 ml of TEOS, 6 ml of ethanol, 1.5 ml of water, 20 µl of the Sc standard solution (100 mg ml^{-1}) and two drops of 0.01 mol dm^{-3} HNO_3 were added. The beakers were put into a thermostated water bath maintained at ~ 75 °C under ultrasonic stirring for ~ 1.5 h to reach the complete gelation. After 20 min., 5 ml of water was added. The gelled materials were dried overnight in an oven at 110 °C. The sol-gel samples were manually ground and pressed into pellets with 12 mm in diameter and 1 mm thickness[10].

I n s t r u m e n t a t i o n

A Jobin Yvon 170 Ultrace with laterally viewed ICP was used for solution analysis. The ICP system combines a monochromator and a polychromator. The monochromator was set on the Pb II 220.353 nm line and other four studied analytes were measured with the polychromator of a Paschen Runge montage using analytical lines Cr II 267.720 nm, Ni II 231.608 nm, Cu II 324.759 nm and Zn I 213.860 nm.

The XRF analysis was performed on an energy-dispersive XRF spectrometer (SPECTRO XEPOS) by polarized radiation, equipped with a Pd X-ray tube and a Si-detector. The real unknown samples were analyzed by the TURBOQUANT method, which is able to analyze the elements Na-U.

The LA-ICP-MS was performed with a laser ablation system UP 213 (New Wave, USA) and an ICP-MS spectrometer Agilent 7500 CE (Agilent, Japan). A commercial Q-switched Nd:YAG laser, operated at the wavelength of 213 nm with the 5th harmonic frequency, was used for ablation. The ablation device is equipped with programmable XYZ-stages that allowed sample movements during the ablation process. The samples were placed into the SuperCell (New Wave, USA) and were ablated by the laser beam, which was focused onto the sample surface through a quartz window. The ablated material was transported by flowing He to the ICP.

Results

The total contents of trace elements were determined using ICP-OES after total decomposition. XRF spectrometry was chosen for result comparison. Relative standard deviation of the total decomposition method and concentration range of tested soils can be found in Table I. Higher RSD value might be due to sample inhomogeneity and uncertainty of the measurement at low element concentration. Satisfactory agreement was found for ICP-OES solution analysis and XRF spectrometry. Correlation coefficient r values exceeded 0.994. Comparison of PN-ICP-OES and XRF determination of zinc as an easily leachable element is presented in Fig. 1.

A five-stage sequential extraction procedure was used for the determination of the speciation of extractable heavy

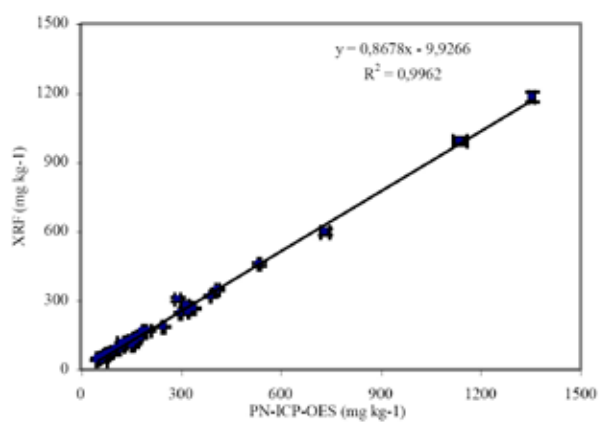

Fig. 1. **Comparison of results obtained by XRF analysis and PN-ICP-OES of soil extracts for Zn I 213.860 nm**

Table III
Comparison of efficiency of extraction by PN-ICP-OES to XRF analysis

Element	Efficiency [%]
Cr	63
Ni	82
Cu	80
Zn	102
Pb	96

metals by PN-ICP-OES. The results as a sum of the five extraction steps were compared with those obtained after total decomposition and by XRF spectrometry. Efficiency of soil extracts to pressed pellets together with correlation coefficients are presented in Table III. The best efficienty of extraction can be observed for lead and zinc. Efficiency of extraction depends on the binding to minerals with different solubility. Low efficiency of chromium can be explained by binding to poorly soluble chromspinels and chromite[12,13].

The soil samples were analysed to evaluate the possibility of quantitative elemental analysis by laser ablation. The results for LA-ICP-MS were compared with the results of XRF as a method representing another type of direct analysis and with the results of solution analysis by ICP-OES as an ordinary method. In the case of Cr, slightly lower concentration values were yielded for the total decomposition using PN-ICP-OES than for both direct analysis methods, which can be caused by the incomplete decomposition of Cr in acids (Fig. 2.).

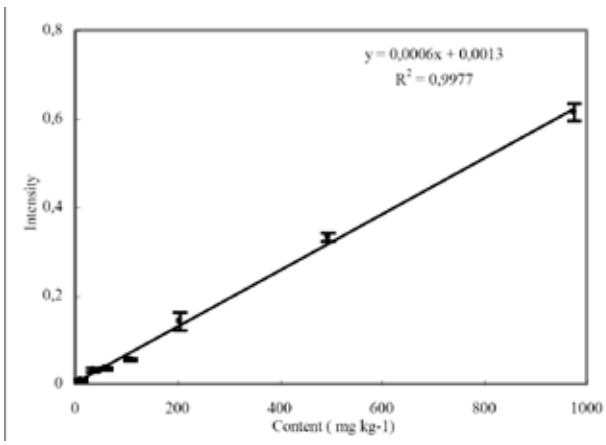

Fig. 2. **Comparison of LA-ICP-MS and PN-ICP-OES after total decomposition results for Cr as a barely leachable element**

The calibration was performed using spiked sample pellets. The soil with a low content of elements of interest was spiked with seven concentration levels to obtain a calibrated range from 24 to 965 mg kg^{-1}. The calibration curves were fitted by a computer program WinStat. All calibration plots were linear over the whole concentration range. Intercepts of the regression lines were tested by t-test and were statistically insignificant ($t_a < t_{0.05;n-2}$) for all studied elements. Correlation coefficient r values were in the range of 0.997–0.999. Calibration line for copper with scandium as internal standard is presented in Fig. 3.

Table IV
Comparison of CRM GBW07407 soil analysis results by LA-ICP-MS and certified values; the uncertainty intervals are calculated on a 95 % confidence level

Method [mg kg^{-1}]	LA-ICP-MS	Certified value
Cr	432 ± 28	410 ± 23
Ni	269 ± 27	276 ± 15
Cu	84 ± 8	97 ± 6
Zn	139 ± 15	142 ± 11
Pb	15 ± 3	14 ± 3

Repeatability of ablation was described as RSD % which was calculated from three measurements of ablation signal on the different locations of each pellet. The RSD values did not exceed 8%.

The accuracy of the method was confirmed by analysis of three CRM soils. Results of CRM GBW 7407 by LA-ICP-MS and certified values are given in Table IV.

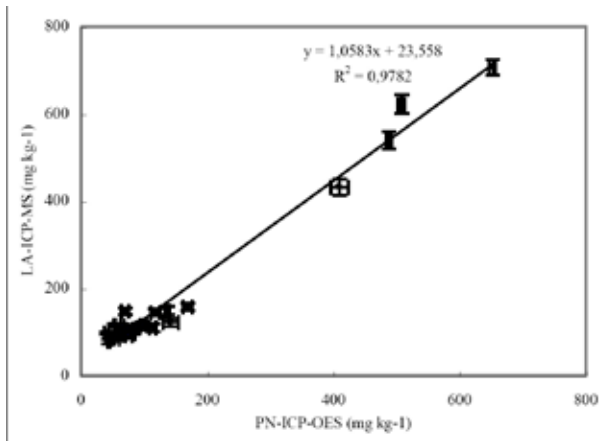

Fig. 3. **Calibration graph for Cu with internal standardization by Sc**

Conclusions

Monitoring of the heavy metals in agricultural soils is gaining the importance because these elements can be accumulated by plants and thus enter the food chain. To obtain total elemental content of Cr, Ni, Cu, Zn, Pb the decomposition with HF and $HClO_4$ by ICP-OES was used. The sequential extraction procedure providing more information than total concentration determination was used to study bioavailability of metals. Methods of total decomposition are very lengthy and sometimes even not fully efficient therefore direct methods are more convenient in such a case. XRF analysis of soil pellets prepared with a wax binder and LA-ICP-MS of perfectly homogenized pellets using sol-gel method represented the direct methods.

The results of direct methods were compared with those obtained by PN-ICP-OES. A satisfactory agreement was

found for all three methods applied to the studied elements, except for chromium as was mentioned.

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L09 TISSUE-SPECIFIC DISTRIBUTION AND ACCUMULATION OF ORGANOCHLORINE POLLUTANTS IN SELECTED RAPTOR SPECIES FROM THE CZECH REPUBLIC

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Introduction

Organochlorine compounds such as polychlorinated biphenyls (PCBs) or organochlorine pesticides (OCPs) are known as persistent organic pollutants (POPs). Despite the ban on production or restrictions on use many years ago the POPs still continue to be found in various samples from the environment and accumulate through the food chains. For their top position within the food chain and the sensitivity to environmental changes birds of prey are very suitable bioaccumulation markers. The amount of pollutants accumulating in the raptors' tissues is related not only to their diet and corresponding trophic position, but also to the differences in accumulation among habitats and ecosystems (aquatic vs. terrestrial)[1]. Moreover, sensitivity also varies greatly between compound and species[2].

However, it is difficult to use birds for the assessment of pollutant transfers within the food web, except for those species partly associated with the aquatic habitats where the transfer can be documented in some measure. For instance the cormorant or heron are fish feeders and top predators in aquatic ecosystems, have relatively high levels of POPs in the adipose tissue, and hence are suitable bioindication species[3].

Because practical and ethical reasons, low numbers and often legal protection inhibit the sacrifice of free-living animals, methods for non-destructive biomonitoring have been developed. Whereas many studies have previously focused on the use of eggs or hair, feathers, on the other hand, have an advantage that they can be collected irrespective of season, age or sex[4]. Another simple approach consists in the use of specimens found dead.

In the Czech Republic, the levels of PCBs in unhatched eggs from raptors were monitored. No intra- or interspecies differences were found and the findings corresponded to those from Germany, Canda or the USA, where the CB 153 was the most abundant congener[5].

This study deals with the levels of organochlorine pollutants in various tissues of raptor species from the Czech Republic and compares the results with those from foreign surveys.

Experimental

The sampling area as well as the raptor and fish species and their detailed description have already been published[6].

Selected tissue samples of investigated specimens were homogenized and dessicated by activated anhydrous sodium sulphate. Two different extraction techniques were employed for the isolation of lipids from the tissues. The samples of muscles, kidneys and liver were extracted by petrolether (SupraSolv, Merck) by means of accelerated solvent extraction (140 °C, 12 MPa, 3 × 5 min static extraction + N_2 purge). The samples of brain, skin, feathers and intestinal content were extracted by petrolether:acetone (1 : 1, v/v) for 6 h in a Soxtec apparatus. The removal of lipids from raw extracts was carried out on an adsorption column packed with Florisil (5 g; 60/100 mesh, Sigma-Aldrich, activated at 600 °C for 6 h). The samples were then eluted with 90 ml of n-hexane: diethylethere (94 : 6, v/v), evaporated to dryness using a rotary evaporator, and dissolved in 1 ml of *n*-hexane.

The quanification of target analytes was carried out by HP 6890N high resolution gas chromatograph with two micro-electron capture detectors (63 Ni m-ECD) and two capillary columns (HT-8 and DB-17ms) operated in parallel (H_2 as the carrier gas). Nine-point calibration curves in a linear range from 0.5 to 1,000 ng ml^{-1} were used and the limit of quantification (LOQ) for all analytes was calculated as 2.5 ng g^{-1} of lipids (i.e. about 0.13 ng g^{-1} d.w. for 4% lipid content in a tissue).

Results

The levels of organochlorine pollutants found in the muscle tissue of cormorants from the Záhlinice area (2007)

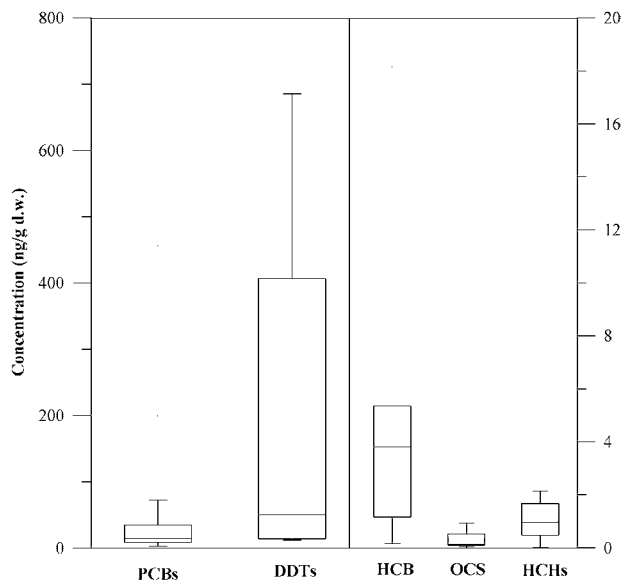

Fig. 1. **Organochlorine pollutants in muscle tissue of common cormorant from the Záhlinice area (n = 8). Horizontal lines represent median, rectangles delimit the 1st and 3rd quartiles, and error bars represent the min/max values. Outlying values are marked as "+" (2007).**

are presented in Fig. 1. PCB congeners 138, 153 and 180, and the p,p'-DDE were the most abundant compounds. As can be read in this graph, there are significant differences in the levels of organochlorines in samples of different specimens. Due to its higher content of lipids, compared with the other tissues, skin was by far "the most contaminated" tissue (approx. five times higher levels of PCBs and OCPs). Extreme values exceeded 5,000 ng g^{-1} w.w. for the sum of 7 PCB congeners, and DDT and its metabolites reached even higher levels. Interspecies differences in the concentrations of POPs are shown in Fig. 2.

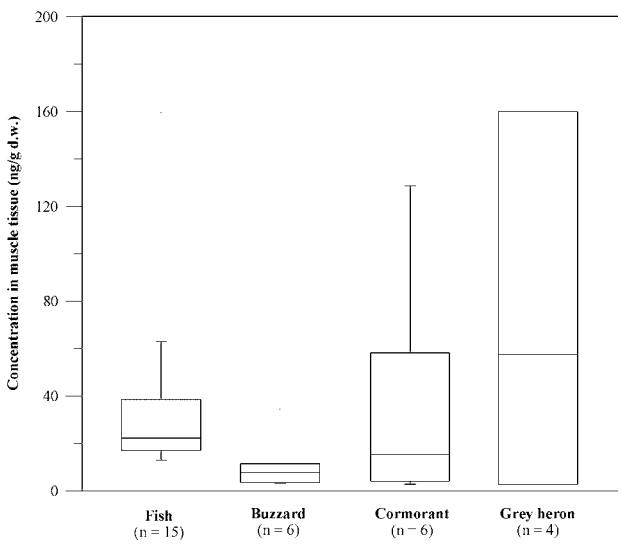

Fig. 2. **PCBs in muscle tissue of species from the Záhlinice area. Horizontal lines represent median, rectangles delimit the 1st and 3rd quartiles, and error bars represent min/max values. Outlying values are marked as "+" (2003).**

Comparison of fish and raptors' tissues from the Záhlinice area showed an increase in the concentrations of POPs towards higher levels of the food chain and the process of accumulation of POPs was clearly illustrated.

Conclusions

Investigated raptor species including cormorant, grey heron and buzzard lived up to their reputation of being the most contaminated members of food chains. Cormorants and grey heron top the aquatic food chain and hence the levels of organochlorines found in their tissues were significantly higher than those in their fish prey. The results can be well compared with other findings not only within the Czech Republic a prove fish and birds of prey to be suitable bioaccumulation markers.

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L11 POTENTIAL APPLICABILITY OF A HIGH PERFORMANCE CHELATION ION CHROMATOGRAPHIC METHOD TO THE DETERMINATION OF ALUMINIUM IN ANTARCTIC SURFACE SEAWATER

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Introduction

In oceanography, aluminium is used as a tracer to fingerprint the location and magnitude of atmospheric dust deposition. Aluminium is particularly suitable as a tracer because of its short residence time in surface seawater, its relatively simple seawater chemistry and the fact that primary input to the open ocean is by atmospheric deposition. The information supplied by surface aluminium concentrations is vitally important to understanding the role that aeolian deposition plays in supplying trace elements to the surface ocean and subsequent effects on biological processes. The information is especially important for furthering knowledge of the biogeochemistry of iron. Iron is of particular interest because it is an essential element for the growth and metabolism of all marine organisms despite only being available in extremely low concentrations (0.1–0.5 nM)[1]. Iron has been shown to limit phytoplankton growth, which in turn may have implications on global climate through drawdown of gases used in photosynthesis, such as carbon dioxide. An accurate and robust method for determining aluminium is thus vital for continuing studies into atmospheric deposition and subsequently climate control.

Flow injection analysis (FIA), has typically been used for the quantification of aluminium in seawater, due to its portability for shipboard use, suitable limit of detection and relative ease of use. However, this technique still requires preconcentration of as much as 10 ml of seawater in order to achieve the required sensitivity[2]. Other common techniques for the determination of trace concentrations of aluminium, such as ICP-MS, are unsuitable for use at sea due to the size of the instrumentation as well as the amount of sample pretreatment required.

Chromatographic techniques have become increasingly popular for the quantification of aluminium in recent years. Ion chromatography has successfully been used for the separation of aluminium in a selection of matrices including natural waters[3–6] and biological samples[7]. The technique is relatively simple and can be coupled with a variety of detection methods such as UV-VIS and mass spectrometry, allowing for both high selectivity and sensitivity.

Chelation ion chromatography (CIC) offers an alternative to traditional ion-exchange IC, particularly for samples of high ionic strength. CIC functions by retaining metal ions according to the stability of the corresponding complexes and allows for the separation and preconcentration of aluminium in complex samples. In addition, CIC also offers the advantages of using only one type of material for both preconcentration/matrix elimination and separation and also the ability to acquire a simplified chromatogram identifying only kinetically-labile and chemically inert species[8,9]. Ion-exchange interactions are likely to occur simultaneously; however, high ionic strength eluents are typically used to ensure chelation is the dominant mechanism.

The iminodiacetic acid functional group has been shown to be a highly promising ligand for the separation of metal ions by CIC[10]. Our previous work has shown the applicability of iminodiacetic acid functionalised silica (IDAS) to the determination of aluminium in paper mill process water[11]. This work detailed the optimised separation conditions of an IDAS packed column including eluent composition, flow-rate and column temperature. Good separation in a complex matrix was achieved, with high column efficiency.

The most commonly used method for the detection of aluminium at low concentrations is the highly sensitive fluorescent detection of its lumogallion complex. This approach has been applied successfully to the determination of aluminium in a wide range of samples, including those with a high salt content, e.g. saline water and body fluids. Nishikawa and co-workers were the first to describe application of the technique to seawater and the batch method has since been incorporated into FIA systems [12]. The limit of detection for the technique has been improved through the addition of surfactants and through optimisation of conditions such as pH and temperature.

This paper is a continuation of our work with IDAS but for the specific purpose of the direct determination of aluminium in seawater. The determination of Al in seawater poses a myriad of potential problems including sample matrix interferences and extremely low concentrations. We have developed a HPCIC method coupled with fluorescent detection of the aluminium-lumogallion complex. The system has been successfully applied to seawater samples obtained in the Ross Sea, Antarctica.

Experimental

A p p a r a t u s

A Metrohm 844 UV/VIS Compact IC was used for all analyses. The system delivered the eluent at 0.3 ml min^{-1} and was set up with a post-column reactor, consisting of a 2 m PTFE reaction coil (1/16" × 0.02"). This reactor was immersed in a water bath for heating above room temperature. Peristaltic pump tubing delivered the PCR reagent at a constant flow-rate of 0.36 ml min^{-1}. A 20 µl sample loop was used unless specified.

A column heater set to 71 °C housed a 200 × 4 mm i.d. column packed with 5 µm IDAS (JPP Chromatography Ltd, UK). Detection was carried out using a Varian Prostar 363 fluorescence detector fitted with a xenon lamp. The excitation and emission wavelengths were set to 500 and 550 nm

respectively. The detector and Compact IC were connected through a Metrohm 830 IC Interface.

Reagents

All reagents were of an analytical grade. A NaCl-HNO_3 eluent (unless otherwise indicated) was made from stock 2M and 1M solutions respectively. All solutions were prepared from a Milli-Q Element purification system, (Millipore, North Ryde, NSW, Australia). A stock 1M MES (Sigma, Castle Hill, NSW, Australia) buffer was made and pH adjusted to 6.05 (unless otherwise stated) with concentrated NaOH. A stock 2M NH_4OAc buffer was prepared from trace metal grade concentrated acetic acid (GFS Chemicals, Powell, Ohio, USA) and ammonia solution (isopiestic distilled concentrated NH_4OH) and pH adjusted to 6.8. A stock 3mM lumogallion (Pfaltz and Bauer, Waterbury, CT, USA) solution was prepared and refrigerated in dark conditions for up to 2 months. Working lumogallion buffers were prepared daily as were aluminium standards.

Samples

Surface seawater was collected aboard the Research Vessel Nathaniel B Palmer (USA) by means of a towed fish. Samples were collected at a depth of approximately 7 m using trace metal clean procedures. The seawater was filtered (0.25 μm) and acidified to pH 2 using trace metal clean HCl.

Results

Separation Conditions

Optimum operating conditions for the separation of aluminium in complex matrices using IDAS have previously been detailed elsewhere[11]. The only modification to these conditions was the use of NaCl rather than KCl in the eluent. The reason for this was the availability of high grade chemical reagent in order to ensure low background fluorescence. In summary these conditions were a 0.25M NaCl–40mM HNO_3 eluent delivered at 0.3 ml min^{-1} with separation on a 200 × 4 mm i.d. column packed with 5 μm IDAS at 71 °C.

Background Fluorescence

A significant dip in fluorescence away from the baseline before the elution of aluminium was observed in preliminary experiments. This dip was up to one fifth the size of the peak of a 0.1 ppm aluminium standard. It was decided that the probable cause was high background fluorescence due to the reagents used to prepare the eluent, in particular the chloride salt. Initially, KCl was used for the preparation of the eluent and despite choosing an analytical grade KCl, the level of aluminium contamination was obviously high. A solution to this was the addition of a trap column positioned before the separation column. The column was packed with Eichrom Diphonix® resin (particle size 100–200 mesh). This resin has diphosphonic and sulfonic acid groups bonded to a polystyrene/divinylbenzene matrix. It is capable of extraction of a range of metals from both neutral and highly acidic solutions. The column, measuring 250 × 4 mm i.d., effectively removed the majority of the aluminium from the eluent, reducing the dip by a factor of 25. In addition, trace metal grade sodium chloride and nitric acid were used in the eluent for subsequent experiments.

Optimisation of Lumogallion Chemistry

Buffer and pH

Work by Howard and co-workers[13] previously reported the optimum pH of the aluminium-lumogallion reaction to be between 4 and 5.5. Resing and Measures later found the maximum response to be in a much narrower range between pH 5 and 5.5(ref.[12]). Based on this fact, MES was chosen as the buffer for initial experiments given its pK_a of 6.27 at 25 °C and subsequent useful buffering range[14]. Although initial chromatograms of a 1 ppm aluminium standard, using a 40mM MES solution at pH 6.2, were promising in terms of sensitivity and efficiency, the pH of the effluent was found to be only 2.9. Increasing the MES concentration to 120mM served to improve this situation, but also resulted in an increase in baseline noise and reduction in both sensitivity and efficiency. Consequently, it was decided to continue investigations using ammonium acetate, a buffer extensively used for the aluminium-lumogallion reaction.

Firstly, the effect of varying the concentration of the ammonium acetate buffer on sensitivity was observed. This was carried out by diluting a stock 3M buffer (pH 6.7) to 0.25. 0.5 and 1 M. The results indicated that a concentration of 0.25 M gave the best result in terms of peak area and also for achieving an effluent pH closest to optimum for the lumogallion reaction. It was shown that peak area of a 0.1 ppm aluminium standard increased almost 1 times through the use of 0.25M compared with 1M ammonium acetate and over 8 fold compared with 40mM MES.

Seawater samples intended for quantification of aluminium require acidification to between pH 1.8–2. Consequently, the buffer utilised in the lumogallion reaction needs to be able to maintain an optimum pH even on mixing with the acidified sample. The 0.25M ammonium acetate buffer was shown to have insufficient buffering capacity when mixed with an acidified sample. Not only did the retention time decrease but a loss in sensitivity also resulted. Given that a decrease in sensitivity was also previously observed with an increase in buffer concentration for ammonium acetate, the only alternative was to increase the pH of the buffer. This was attempted but it appeared that even increasing the buffer pH to 8 resulted in little improvement. This meant that short of sacrificing sensitivity for buffering capacity, ammonium acetate was not the best choice for the analysis of acidified seawater.

The choice of buffers capable of maintaining a pH of approximately 5.5 is fairly limited. This led to the decision to reinvestigate MES. For comparative proposes, a 0.25M solution of MES (pH adjusted to 6.05 with NaOH) was firstly trialed. The result was an equivalent sensitivity to ammonium acetate but with the added advantage of no loss in sensitivity

between acidified and non-acidified samples. Similarly, changes in retention times were negligible. The remaining issue with the use of MES was the increase in baseline noise and subsequent increase in detection limits. This problem was overcome by pre-cleaning the buffer using a column packed with Eichrom Diphonix® resin. The resulting baseline noise reduced approximately three times and the corresponding background fluorescence was almost seven times less.

It was thus determined that a pre-cleaned buffer of 0.25M MES adjusted to a pH of 6.05 with NaOH, was the optimum choice for the determination of aluminium in acidified seawater samples.

Temperature

The response of the reaction between aluminium and lumogallion has been investigated in both batch techniques and flow systems. In the batch method, an optimal temperature of 80 °C is generally accepted[13,15], whereas FIA methods tend to use 50 °C. The latter is based on investigations carried out by Resing and Measures which concluded that most of the temperature-based reaction rate gain had been achieved by this temperature[12]. Independent investigation into the effect of temperature on the rate of reaction was undertaken by us due to the fact a different buffer was used. It was found that the highest response, in terms of peak area, was obtained at temperatures between 65 and 75 °C (Fig. 1.). Based on this response, 70 °C was chosen as the temperature at which to operate the post column reactor for all subsequent anlyses.

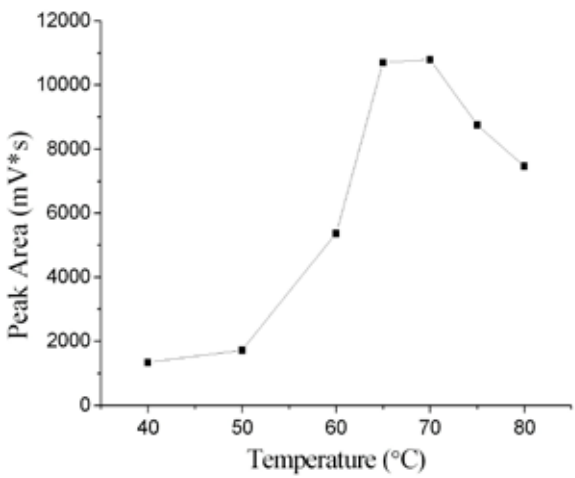

Fig. 1. **Dependence of fluorescence response on temperature**

Lumogallion and Reaction Coil

The extent of chemical reaction needs not be complete for an analytical technique to be valid. However, it is desirable to obtain as high a reaction yield as possible in order to ensure the technique has good precision. For the reaction between aluminium and lumogallion, the concentration of post-column reagent may be changed, along with temperature and reaction time, in order to control the extent of reaction. Three concentrations of lumogallion (0.03, 0.04 and 0.05 mM) were tested in order to exhaust possible improvements to the system via this approach. The concentrations chosen were based on those used in flow systems. It was found that at concentrations higher than 0.03 mM, no significant improvements were achived. Additionally, the effect of increasing the length of the post column reaction coil from 2m to 4m was also studied. The result, however, was a slight reduction in fluorescence. A MES buffer containing 0.03 mM lumogallion together with a 2m reaction coil were thus used in all subsequent analyses.

Surfactant

Howard and co-workers reported an increase in the fluorescence intensity of the aluminium-lumogallion complex of as much as 5-fold through the addition of a non-ionic surfactant[13]. Further investigation has been carried out by Resing and Measures[12], which showed that Brij-35 enhanced fluorescence to a greater extent than other surfactants, such as Triton X-100 and cetylammonium bromide (CTAB). In order to ensure the lowest limit of detection was achieved for this system, an investigation into the effect of surfactants was also carried out. The results differed substantially from those discussed earlier. It was found that although the addition of Brij-35 enhanced fluorescence marginally, a simultaneous increase in baseline noise negated any improvement achieved. Interestingly, when CTAB was tested, the aluminium peak disappeared altogether. This was considered to be an effect of the surfactant adhering to the tubing walls and effectively stripping the aluminium from the reagent stream. The system required flushing with methanol in order to resume normal operation. Consequently, further investigation into the possible use of surfactants was abandoned, with the decision to explore other approaches to lowering the detection limit being deemed more favourable.

S a m p l e V o l u m e

A more attractive approach for achieving a low LOD was increasing the sample loop volume. All previous experiments had been carried out using a volume of 20 µl. The response of the system to higher volumes was investigated and the results are depicted in Fig. 2. It can be seen that for volumes between 20 and 500 µl, the system follows a linear response, as expected. It was also noteworthy that no reduction in column efficiency was experienced at higher volumes. The highest efficiency was achieved for a 100 µl sample loop, which was unexpected considering that band broadening is generally associated with increased sample size and is often responsible for an observed reduction in performance of the chromatographic column as injection volume is increased.

Another unexpected result of increasing the sample volume was an increase in retention time. Generally, a decrease in retention time would be expected due to competition from other analytes for chelation sites, especially in such a complex matrix as seawater. This was shown not to be the case for IDAS and may be explained in terms of the forma-

tion of negatively charged aluminium complexes in seawater (e.g. fluoro complexes) and the high ionic strength of the eluent. The effect of ionic strength has been reported to affect the retention of ions in chelation IC[8,16]. In an environment of sufficiently high ionic strength the repulsion between negatively charged aluminium species and the iminodiacetic acid functional groups may be reduced. This can result in a subsequent increase in retention time as observed in our studies. This response is actually considered favourable as it allows for additional stabilisation of the baseline between the minor dip in fluorescence and elution of the aluminium.

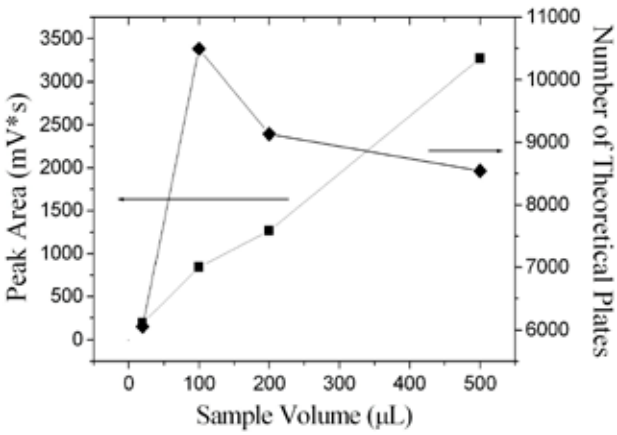

Fig. 2. **Effect of increasing sample volume on column performance and fluorescence response**

Seawater Samples

At this stage, the optimised HPCIC system coupled with fluorescence detection had been shown to be applicable to the determination of aluminium in acidified standards prepared in Milli-Q water. Previous work by us has shown that IDAS can be successfully applied to the analysis of samples with a complex matrix but it had not yet been used for the detection of aluminium in seawater. Seawater is difficult to analyse not only in terms of the high salt content, but also due to the number of other potentially interfering ions, such as iron and magnesium. However, preliminary chromatograms showed no co-elution problems and there was only one additional peak (at ~ 8 min) other than aluminium. Based on previous findings this peak is likely to be due to iron and/or a mixture other analytes e.g. sodium and calcium.

Calibration of the system using a 500 µl sample loop was carried out by means of standard addition to an Antarctic seawater sample containing low levels of aluminium. The limit of detection was determined from the standard deviation of clean seawater and determining the signal equivalent to three times this value (i.e. 3σ). A LOD of 0.39 nM was achieved using a 500 µl sample loop. Good linearity of the system was observed between 1.8 and 36 nM.

Chromatograms of Antarctic seawater for different injection volumes are given in Fig. 3.

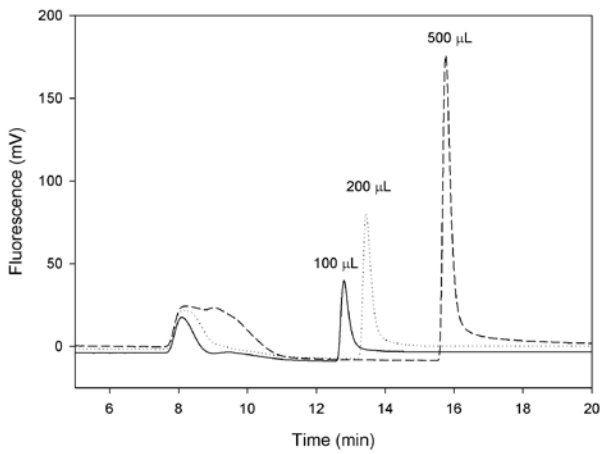

Fig. 3. **Chromatogram of Antarctic seawater for different injection volumes**

Conclusions

The optimised HPCIC system with fluorescence detection of the aluminium-lumogallion complex shows promise for the quantification of aluminium in Antarctic seawater. The IDAS chromatographic column does not suffer from issues such as co-elution of species with aluminium and produces peaks of good efficiency in a reasonable timescale. The response of the system to standard addition is linear and is applicable over a concentration range valid to seawater analysis. Additionally, the LOD achievable with the system means it should be capable of handling the low concentrations of aluminium expected in Antarctic seawater.

At this stage, the system has not been successfully applied to the quantification of aluminium in a seawater sample. The method currently suffers from an enhanced fluorescent response most likely due to matix interferences of seawater. It is difficult to determine the extent of this enhancement since there is not a certified seawater reference material for aluminium; but it is believed that, at present, the system produces a response approximately three times higher than the true value. Studies are underway to eliminate this problem and are currently focusing on the removal or adequate separation of aluminium from the remainder of the seawater matrix.

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L12 DIRECT ANALYSIS IN REAL TIME – TIME-OF-FLIGHT MASS SPECTROMETRY: ANALYSIS OF PESTICIDE RESIDUES AND ENVIRONMENTAL CONTAMINANTS

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Introduction

Ambient desorption ionization mass spectrometry (MS) is a rapidly growing area representing an attractive alternative to conventional analytic approaches. Recently introduced ionization techniques, such as direct analysis in real time (DART)[1], desorption electrospray ionization (DESI)[2] or atmospheric pressure solids analysis probe (ASAP)[3], allow direct examination of various types of samples in the open atmosphere and at ground potential. Little or no sample treatment prior to analysis is required. Additionally, time-consuming separation of sample components, which is usually employed by chromatographic methods, can be omitted with ambient MS.[4]

The ionization process with DART is based on interactions of metastable atoms of gas with atmosphere (H_2O, O_2) and sample components. The gas (usually helium) flows through a tube divided into several compartments. In a discharge chamber, ions, electrons and metastables are formed. In the next step, charged species are removed from the gas stream and heated gas promotes the desorption process. Ionization of the sample occurs in the area between the ion source and a mass spectrometer inlet (sampling gap). DART provides relatively simple mass spectra characterized mainly by $[M + H]^+$ and $[M]^+$ in positive-ion mode or $[M-H]^-$ and $[M]^-$ in negative-ion mode.[1] It is worth to notice, that DART technique has common features with atmospheric pressure chemical ionization (APCI) as the formation of metastables take place in an electrical discharge.[1,4]

DART ion source can be hyphenated to any type of mass spectrometer. However, when coupled to a high-resolution time-of-flight mass spectrometer (TOFMS), accurate mass measurement is enabled, allowing the confirmation of target analyte identity and calculations of elemental compositions of "unknowns". For correct identification of "unknowns", it is essential to gain knowledge about the examined matrix to allow discrimination of potential compounds suggested by the software.

Until now, very few papers dealing with applications of DART have been published.[5-9] In following examples, the potential of DART–TOFMS technique for qualitative and quantitative analysis of *(i)* pesticide residues, in particular case, strobilurins in wheat grains, *(ii)* thiabendazole on cut-flower leaves, and *(iii)* rapid screening of brominated flame retardants (BFRs) in in-door dust extract, will be demonstrated.

Experimental

C h e m i c a l s

Pesticide standards (≥ 99 %) were obtained from Dr. Ehrenstorfer (Germany), decabromodiphenyl ether (BDE-209) standard (≥ 98 %) was provided by Cambridge Isotope Laboratories (USA). Solvents used for sample extractions and preparations of standard solutions were HPLC-grade. Poly(ethylene glycol) 600 was from Sigma-Aldrich (Germany), anhydrous Na_2SO_4 was supplied by Merck (Germany).

S a m p l e P r e p a r a t i o n

(i) An amount of 12.5 g of milled wheat grains was spiked with an internal standard (prochloraz) at a concentration of 250 ng g^{-1} and extracted by shaking with 50 ml of ethyl acetate and 10 g of anhydrous Na_2SO_4. The suspension was filtered and the volume was reduced by evaporation to 25 ml. Similarly, wheat grain extracts spiked with strobilurins (azoxystrobin, kresoxim methyl, pyraclostrobin, trifloxystrobin, dimoxystrobin and picoxystrobin) in the range from 12 to 1200 ng g^{-1} were prepared. Wheat grains with incurred residues of azoxystrobin, kresoxim methyl and pyraclostrobin (reference material) were processed as described above.

(ii) Flowers (roses) were purchased from local florists shop. The leaf was separated from the rest of flower and its surface was directly analyzed.

(iii) In-door dust containing BFRs (mainly BDE-209) was extracted using ASE 300 pressurized liquid extraction system (Dionex, USA): a hexane–acetone (1 : 1, v/v) mixture was used for extraction. The residues of extract were dissolved in isooctane.

D A R T – T O F M S A n a l y s i s

For DART–TOFMS analyses, the system consisting of a DART ion source (IonSense, USA), a JEOL AccuTOF LP high-resolution mass spectrometer [JEOL (Europe) SAS, France], and an AutoDART HTC PAL autosampler (Leap Technologies, USA) was used. Helium gas was flowed at 2.9 dm^3 min^{-1}, discharge needle voltage was ± 3000 V, while perforated and grid electrode voltages were set to ± 150 V and ± 250 V, for positive and negative-ion mode, respectively. Other system parameter settings were changed depending on examined analytes, as summarized in Table I. To monitor bromine fragment ions originated from BDE-209, the cone voltage of the mass spectrometer was adjusted as described in results section.

Automated introduction of liquid samples was carried out with the use of Dip-it™ tips (IonSense, USA). Solid samples (flower leaves) were introduced manually by placing them in front of DART source. Poly(ethylene glycol) 600 solution (200 μg ml^{-1}) was introduced at the end of each sample analysis to perform internal mass calibration (mass drift compensation). The mass resolution of the instrument

Table I
DART–TOFMS parameter settings

Analytes	Polarity	Beam temp. [°C]	Ion guide voltage [V]
Strobilurins	Positive	300	1000
Thiabendazole	Positive	150	800
BDE-209	Negative	300	600
Bromine ions	Negative	350	400

during the measurements was typically 6,000 full width at half maximum (fwhm).

Results

Analysis of Strobilurins

The strobilurins and prochloraz (internal standard) were detected as $[M + H]^+$ ions (see Fig. 1.). A high mass resolving power of TOFMS instrument enabled the identity confirmation of target analytes on the basis of elemental composition calculations; the differences between measured (accurate) and calculated (exact) masses ranged from –2.27 to 5.10 ppm.

Fig. 2. shows the total ion current (TIC) record of six injections of wheat grain extract spiked with strobilurins (240 ng g^{-1}) and prochloraz (250 ng g^{-1}). Unfortunately, the

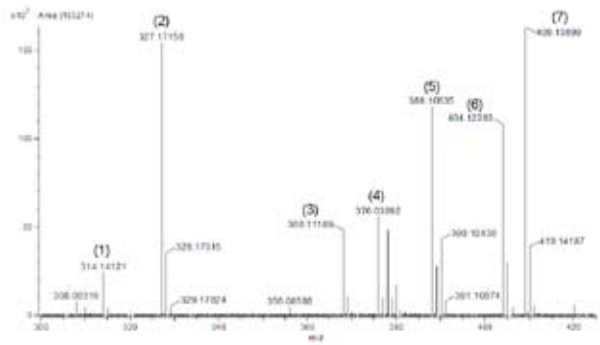

Fig. 1. **Dart positive mass spectrum of wheat grain extract spiked with strobilurins (240 ng g^{-1}) and prochloraz (250 ng g^{-1}); (1) Kresoxim methyl, (2) Dimoxystrobin, (3) Picoxystrobin, (4) Prochloraz, (5) Pyraclostrobin, (6) Azoxystrobin, (7) Trifloxystrobin**

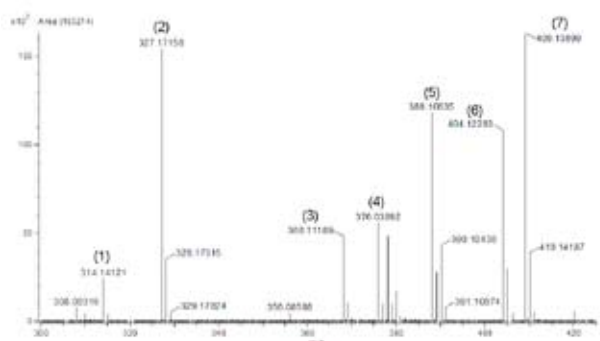

Fig. 2 **TIC chromatogram of six repeated wheat grain extract introductions [(1)–(6)] followed by PEG 600 solution (7)**

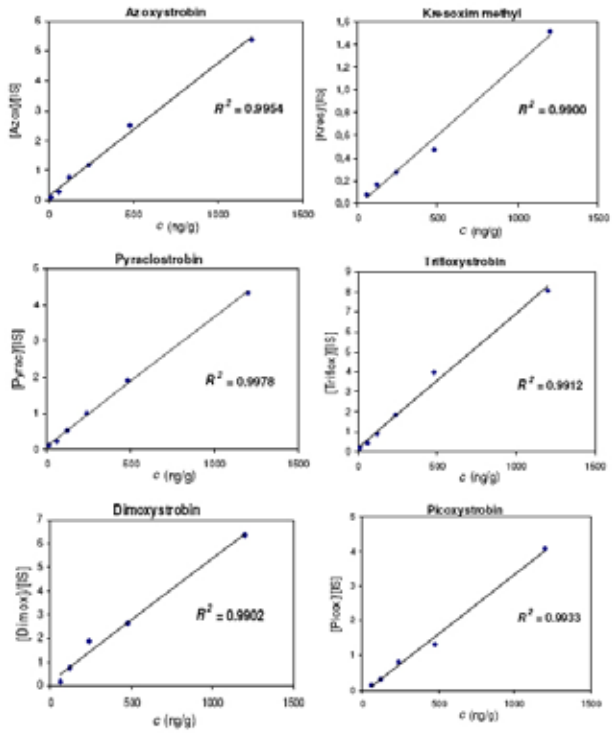

Fig. 3. **Examples of calibration curves of matrix-matched strobilurin standards**

absolute response of the detector, even when employing autosampler, was poorly repeatable because of the dependence of position of the sampling tip and the sampling gap. Therefore, an internal standard had to be used for quantification of strobilurin residues. Calibration plots obtained by analyses of matrix-matched standards (see Fig. 3.) were constructed by plotting the ratio of analyte/internal standard ion intensity *vs.* concentration of particular analyte. Acceptable linearity was obtained for tested concentration range, regression coefficients of calibration curves were higher than 0.99.

In the next step, basic performance characteristics of the method were estimated using spiked samples. The repeatability was in the range from 8 to 15 % (n = 6, 60 ng g^{-1}), LOQs (limits of quantification) ranged from 12 to 30 ng g^{-1}. Considering the European regulation requirements, this method can be useful for rapid control of strobilurin residues in wheat grains[10]. For comparative purposes, wheat grain sample containing incurred residues of strobilurins was analyzed using

Table II
DART–TOFMS and LC–MS/MS methods: Analysis of incurred residues in wheat grains

Analyte	Concentration [ng g^{-1}]	
	DART–TOFMS	LC–MS/MS
Azoxystrobin	445	429
Kresoxim methyl	45	52
Pyraclostrobin	202	190

in-house validated method employing liquid chromatography–tandem mass spectrometry (LC–MS/MS). A good agreement of the results generated by two alternative approaches is documented in Table II.

Direct Detection of Thiabendazole in Plant Leaf

In this experiment, the possibility to monitor pesticide residues directly from the surface of the flower leaf was examined. For this purpose, the temperature of gas beam was decreased to 150 °C. Fig. 4.(A) shows positive mass spectrum of the leaf surface obtained by DART–TOFMS. In zoomed mass spectrum (Fig. 4.(B)), ion m/z 202.04410 corresponding to protonated thiabendazole molecule $[C_{10}H_8N_3S]^+$ (theoretical mass m/z 202.04389) was observed. No other pesticide compounds were detected in examined sample.

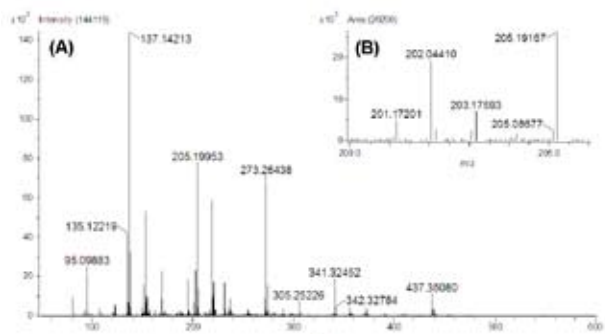

Fig. 4. **DART positive mass spectrum of flower leaf; (A) m/z 50–600, (B) m/z 200–206. The ion m/z 202.04410 corresponds to thiabendazole**

Screening of BFRs in In-Door Dust

The most common methods used in analysis of BFRs employ gas chromatography coupled to mass spectrometry (GC–MS) operated in negative chemical ionization mode (NCI)[11]. The ions $[^{79}Br]^-$ and $[^{81}Br]^-$ are typically the base peaks in NCI mass spectra of these compounds and due to their selectivity they are frequently used for quantification purposes[12]. Supposing some similarity of BFRs fragmentation under NCI conditions in GC–MS and negative APCI, DART–TOFMS was proposed as a suitable approach for rapid screening of BFRs.

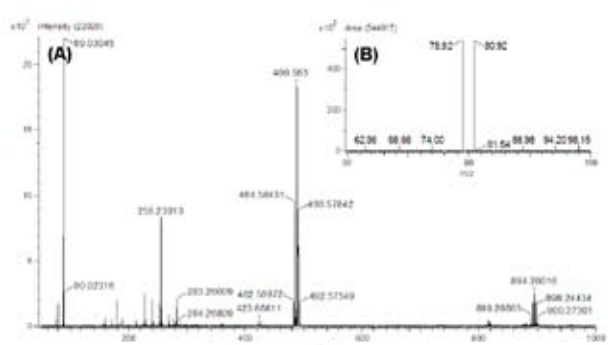

Fig. 5. **DART negative mass spectrum of BDE-209 standard solution (50 μg ml^{-1}); (A) beam temp.: 300 °C, cone volt.: –20 V, (B) beam temp.: 350 °C, cone volt.: –140 V**

In the first phase of this experiment, the ionization of BDE-209 by DART was investigated. As documented in Fig. 5.(A), phenolate anions resulting from the cleavage of the ether bridge and anions resulting from bromine abstraction were observed after introduction of BDE-209 standard solution. To induce fragmentation, the cone voltage was decreased from -20 V to -140 V. Under these conditions, intensive $[^{79}Br]^-$ and $[^{81}Br]^-$ ions were the only ions in recorded mass spectrum (Fig. 5.(B)).

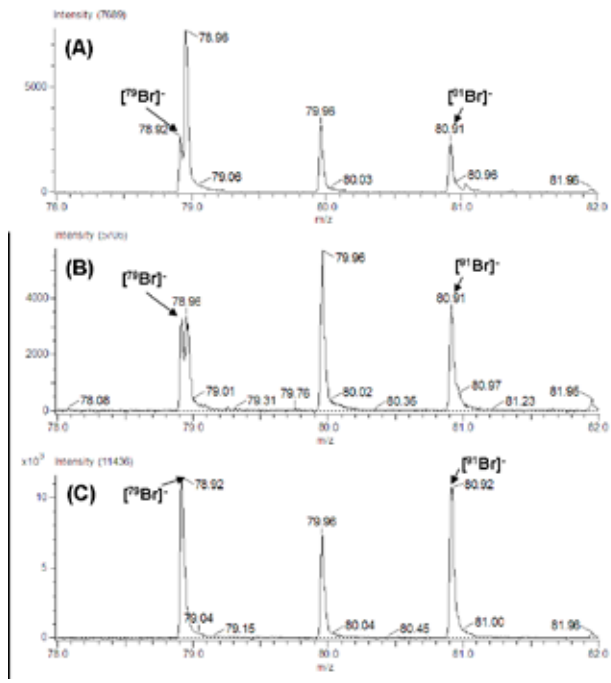

Fig. 6. **DART negative mass spectrum of in-door dust extract; (A) cone volt.: –140 V, (B) cone volt.: –200 V, (C) cone volt.:–240 V**

While it was not possible to detect BFRs in dust extract due to high chemical noise, both bromine ions were distinctly recognized when fragmentation was induced (Fig. 6.(A)). To remove interfering ion with a mass close to $[^{79}Br]^-$, an attempt to induce its fragmentation was undertaken. As shown in Fig. 6.(B) and Fig. 6.(C) this was achieved by further decrease of cone voltage value.

Conclusions

DART–TOFMS technique can be used for determination of strobilurin fungicides in milled wheat grain extracts obtained by simple extraction procedure without time-consuming chromatographic separation. This method withstands the regulation demands of the European Union for the control of pesticide residues; moreover, simplified workflow enables examination of many samples within a short time period.

Qualitative analysis of solid samples without any sample preparation is a challenging application of this novel technique. DART–TOFMS was shown to be a useful tool enabling rapid examination of plant surface and detection of pesticide used for flower treatment.

Preliminary results indicate the potential to introduce new concepts into rapid screening of BFRs by employing DART–TOFMS. In addition, the information provided by both negative and positive mass spectra should be exploited with the aim to detect the presence of other contaminants. Of course, more follow-up research is needed, with a special focus on quantification of target compounds and identification of unknowns.

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L13 APPLICATION OF NEEDLES AS BIOINDICATORS FOR THE EVALUATION OF PERSISTENT ORGANIC POLLUTANTS ENVIRONMENTAL CONTAMINATION LEVEL

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Introduction

Bioindicators are living organisms in which concentrations of organic pollutants considerably exceed those found in air, water, sediments, or soil. Bioindicators, which are frequently used in monitoring studies and screenings, should allow selective and specific determination of contaminants not only in all compartments of the environment, but also in all links of food chains of species living in the area under study. Contaminants detectable by the use of bioindicators include also PCB indicator congeners 28, 52, 101, 118, 138, 153, 180 which rank with priority pollutants monitored in the Czech Republic[1]. Plant bioindicators are used in environmental studies of agrarian ecosystems in our country where they can yield information for both conventional monitoring and biomonitoring. The most frequently used plant species are alfalfa, cereals, and oil plants[2]. The source of contamination is of great importance. Monitoring of PCBs can often identify long-distance transport as one of the contamination sources. Airborne volatile PCBs can originate from various sources including agricultural production[3]. Thus, PCBs penetrate into plant tissues and influence the contamination level. Papers dealing with the contamination of crops by xenobiotics are rather scarce. Most of the respective investigations were carried out in fodder plants and were oriented rather on effects of feeding of contaminated crops to farm animals[1].

Of all above-mentioned plant bioindicators, coniferous plants except for larch have the greatest informative value when the leaf analysis method is used. Needles do not fall off every year as compared to deciduous trees, and one may monitor a degree of burden using different methods such as the discoloration of assimilatory organs, sudden changes in coloration, excessive leaf-fall, crown thinning, partial or complete dieback of trees, and particularly the above-mentioned methods of leaf analysis.

Knowledge of the level of contamination of this link of the food chain is therefore necessary for studies of xenobiotic transfer[1,4]. Comprehensive studies of plant contamination were completed in Moravian areas affected by disastrous floods in 1997 and 1998. Effects of floods on the contamination of soil and vegetation by persistent organic substances are summarized in the „Report on the 1998 Monitoring Results - Hazardous Substances within Food Chains and Influencing Imputes published by the Ministry of Agriculture of the Czech Republic in 1998[2].

Synthetic xenobiotics are included in persistent organic pollutants (POPs) group; they represented a significant risk to the environment owing to their physico-chemical and toxicological properties.

PBDEs are aromatic substances whose structures resemble that of PCBs (see Fig. 1.).

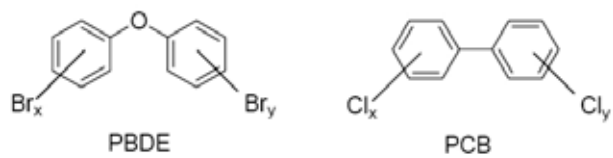

Fig. 1. **PBDEs and PCBs**

The numbering of individual PBDE congeners, whose total sum is 209, complies with the IUPAC nomenclature used in the numbering of PCBs.

Physicochemical Properties of PBDEs

Tri- (major congener 28), *tetra-* (47), *penta-* (99, 100), *hexa-* (153, 154), *hepta-* (183) and *deka-*(209) are the most commonly used PBDE groups which also occur most frequently in the environment.

PBDEs are lipophilic and persistent substances that show low solubility in water. Because of their high resistance against acids, bases, heat, light, and redox reactions, they pose a significant risk to the environment. When they enter the environment, they remain there for a prolonged period of time due to their physical-chemical properties. The octanol/water partition coefficient ($\log K_{ow}$) is another important characteristic of these compounds. The values of their $\log K_{ow}$ vary in a range of 5.98 (28)–9.97 (209), which indicates that these substances are highly hydrophobic.

Upon excessive heating and burning, PBDEs will decompose to very toxic substances such as polybrominated dibenzo-p-dioxins (PBDD) and dibenzofuranes (PBDF). The melting point of PBDEs varies from 64 °C (BDE 28) to 302.5 °C (BDE 209) whereas many congeners are liquids at standard conditions.

PBDEs are used as fire retardants. In this application the ideal situation is when a retardant decomposes at a temperature by about 50 °C lower than that of a polymer – PBDEs meet this requirement with a number of polymers.

Production

The industrial synthesis of PBDEs usually proceeds through catalytic reaction between a diphenyl ether and bromine, yielding a mixture of different isomers. Alternatively, PBDE may also be prepared from phenolate and bromobenzene or by allowing diphenyliodonium salt to react with bromophenolate.

Novel synthetic procedures have been recently developed. One of the most significant developed procedures uses 2,5-dibromo-4-fluoronitrobenzene as a precursor to produce congeners 153 and 154 or employs a modified Sinaki reaction to prepare congener 81.

Industrial Use of PBDEs

DekaBDE

This compound is extensively used in "high-impact" polystyrenes, thermoplastic polyester resins, acrylonitrile-butadiene-styrene rubber, nylon, PVC, and elastomers. BDE 209 is also widely used in isolation materials for electric cables. There is a large number of commercial products based on BDE 209 such as widely used Bromkal 81, DE 83, FR-1210, Chemflam 011, Hexcel PF1.

***Okta*BDE**

*Okta*BDE congeners are mainly used in computer components and the components of office instruments. Furthermore, they are also known to be used in various thermoplastics, adhesives and coating compositions. Bromkal 79-8 DE, FR 143, Tardex 80, etc. are commercial names of widely used mixtures.

***Hexa*BDE**

*Hexa*BDE together with *penta*BDE congeners are contained in commercial mixtures (for example BR 33 A).

***Penta*BDE**

Commercial mixtures are used as additives in epoxy-resins, phenol resins, and textiles. This particularly applies to DE 71, FR 1205/1210, Bromkal 70, Bromkal 61. The Bromkal 70-5 DE mixture (34 % *tetra*BDE, 60 % *penta*BDE and 6 % *hexa*BDE) was widely used in the past.

***Tetra*BDE**

T*etra*BDE congeners have identical applications as *penta*BDE. The average levels of congeners in the commercially used mixture are as follows: 41 % of *tetra*BDE congeners, 45 % *penta*BDE congeners, 6 % *hexa*BDE congeners, and about 8 % of PBDE of unknown structure. Some groups of BDEs are not produced individually but occur as impurities in commercial mixtures (for example nonaBDE or heptaBDE). The least brominated classes of congeners such as triBDE, diBDE, and monoBDE, are neither produced commercially, nor used otherwise.

General use of BDEs

More than one half of PBDEs is used in the production of electric and electronic devices and components, with *Deka*BDE, *Okta*BDE and *Penta*BDE being used most widely, as mentioned above. The highest levels of PBDEs occur in domestic appliances. The reason is that the devices of this kind (TV sets, vacuum cleaners) contain the highest portion of plastics, as compared to other above-mentioned classes of devices, with fire retardants being usually bound to such plastic materials.

Experimental

Samples were taken in several locations. Organochlorine pesticides (DDT and its metabolites, γ-HCH and HCB), polychlorobiphenyls (PCB) and polybromodiphenylethers (PBDE) were selected for analysis. The isolation of tracked analytes from plant matrices was performed by the means of extraction processes, namely via sonication and Soxhlet extraction, extract purification was realized by column chromatography. The final determination was performed by gas chromatography with μECD detection and the confirmation analyses by gas chromatography and mass spectrometric detection.The procedure is summarized in following Scheme 1.

Table I
Polychlorinated biphenyls in needles

	Concentration [ng g^{-1}]						
	CB 28	CB 52	CB 101	CB 118	CB 138	CB 153	CB 180
Pine	0.25	0.10	0.07	0.06	0.06	0.07	–
Fir	0.33	0.12	0.09	0.10	0.07	0.12	0.05
Blue Spruce	0.30	0.09	0.07	0.08	0.10	0.09	0.04

Table II
Organochlorinated pesticides in needles

	Concentration [ng g^{-1}]							
	4,4'-DDT	4,4'-DDE	4,4'-DDD	2,4'-DDT	2,4'-DDE	2,4'-DDD	γ-HCH	HCB
Pine	0.08	0.17	<LOQ	<LOQ	ND	ND	0.15	0.15
Fir	0.21	0.24	0.07	0.14	<LOQ	ND	0.12	0.09
Blue Spruce	0.11	0.11	0.04	0.11	ND	ND	0.13	<LOQ

Table III
Polybrominated diphenyl ethers in needles

	Concentration [ng g^{-1}]									
	BDE 3	BDE 15	BDE 28	BDE 47	BDE 99	BDE 100	BDE 118	BDE 153	BDE 154	BDE 183
Pine	0.80	0.40	4.50	1.50	ND	<LOQ	1.20	1.00	1.60	1.70
Fir	1.30	ND	2.20	<LOQ	0.70	ND	ND	<LOQ	ND	ND
Blue Spruce	0.50	ND	1.80	1.40	2.50	1.20	0.80	0.80	2.10	<LOQ

Collecting needles
(10 g each sample)
↓
Grounding/extraction
3 × 20 min. with n-hexane-acetone (3 : 2)
↓
Clean-up on Florisil column
elution with n-hexane-acetone (94 : 6)
↓
GC/μECD analysis of extracts

Scheme 1

Results

The study submitted deals with problems of the utilization of spruce, pine and fir needles for the evaluation of ecosystems' primary organic pollutants (POPs) load.

Tables I, II and III show the obtained results – concentrations of polychlorinated biphenyls, organochlorinated pesticides and polybrominated diphenyl ethers in needles, respectively.

On the basis of acquired results, it could be stated that level of needles contamination is not negligible. Within the group of PCBs the highest concentrations were found at PCB 28 with highest volatility. Pesticide 4,4'-DDT and 4,4'- DDE, γ-HCH and HCB were dominant from study groups of organochlorinated compounds. It was surprise that PBDE levels were considerably higher than those of other POPs, especially BDE 28.

Conclusions

Needles of pine, fir and blue spruce were used as bioindicators of the atmospheric contamination by persistent organic pollutants. These needles are covered by waxy layer, which effectively concentrates the vapour phase pollutants from surrounding atmosphere. The results proved the suitability of the conifera needles for the estimation of atmospheric contamination by persistent organic pollutants.

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2.2. Posters

P01 ULTRATRACE DETERMINATION OF SILVER IN PRECONCENTRATED WATER SAMPLES BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY

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Introduction

Silver is one of the industrially important elements. It is used for the preparation of corrosion-resistance alloys and its compounds are extensively used in the processing of foods, drugs and beverages and in filters and other equipments to purify water. It also has an important role in electrical and electronic application, photographic film production and the manufacturing of fungicides[1,2]. These widespread applications have resulted in increased silver content of environmental water samples. In turns, owing to the toxicity of silver to many aquatic organisms even at low concentrations, the serious environmental problems may occur. Therefore, simple and highly sensitive methods are needed to monitor the Ag levels in water samples at ever decreasing concentrations.

Several atomic spectrometric techniques such as flame and electrothermal atomic absorption spectrometry (FAAS and ETAAS) [3,4], inductively coupled plasma atomic emission spectrometry (ICP-AES)[5] and inductively coupled plasma mass spectrometry (ICP-MS)[6] have been proposed for the determination of silver in different environmental samples. In order to improve the detection limit, various preconcentration procedures have also been used in combination with the above-mentioned techniques. These include solvent extraction[7], solid phase extraction[8], precipitation[9], adsorption on tungsten wire [10] and cloud point extraction[11]. However, most of these procedures are laborious, time-consuming and may cause sample contamination.

Recently, Assadi and co-workers introduced a novel microextraction method called dispersive liquid–liquid microextraction (DLLME) as a highly sensitive, efficient and powerful method for the pre-concentration and determination of traces of organic and inorganic compounds in water samples[12,13]. In the present work, the DLLME was combined with ETAAS for determination of silver for the first time. In this method, an appropriate mixture of extraction solvent and disperser solvent is injected rapidly into an aqueous sample containing silver ions and sodium diethyldithiocarbamate (DDTC) by a syringe. Then, the resulting cloudy solution is centrifuged and the fine droplets sedimented in a few-µl volume at the bottom of the conical test tube are finally introduced into the ETAAS for the determination of its silver content. The applicability of this approach was validated for the determination of silver in water samples. The proposed method was also applied to the determination of silver in several water samples with satisfactory results.

Experimental

Reagents and Solutions

Reagent grade carbon disulfide, carbon tetrachloride and chloroform, as extraction solvents, and acetone, acetonitrile, methanol and ethanol as disperser solvents from Merck chemical company. Doubly distilled deionized water was used throughout. Analytical grade nitrate salts of silver and other cations (all from Merck) were of the highest purity available and used without any further purification except for vacuum drying. The stock solution of silver (1,000 mg dm^{-3} for atomic spectroscopy standard) was prepaed by dissolving 0.1575 g of silver nitrate (Merck) in deionized water containing 1 ml concentrated nitric acid (Merck) in a 100 mlvolumetric flask and diluting to mark with deionized water and stored in the dark. Working standard solutions were prepared by serial dilutions of the stock solution with ultrapure water prior to analysis. The chelating agent, 0.001M sodium diethyldithiocarbamate (DDTC) solution was prepared daily by dissolving the appropriate amount of DDTC (analytical grade, Merck) in methanol (suprasolv, Merck).

Tap, underground and river water samples used for development of the method were collected in PTFE containers from the Tehran and added appreciated amount of HNO_3 to adjust pH 3 and stored in dark at 4 °C and analyzed within 48 h of collection without previous treatment or filtration.

Instrumentation

The experiments were performed using a Perkin Elmer atomic absorption spectrometer (AA 1100B), equipped with a graphite furnace atomizer HGA-700. Deuterium background correction was employed to correct non-specific absorbances. All measurements were performed using the peak height. An Intensitron™ silver hollow cathode lamp and a pyrolytic coated graphite tube (Perkin Elmer) were used. The sample injection volume was 10 µl in all experiments. The instrumental parameters and temperature program for the graphite atomizer are listed in Table I. Argon gas with 99.95% purity was purchased from Roham Gas Co. (Tehran, Iran) was used as protected and purge gas. A Kendro 1020D centrifuge (Germany) was used for centrifugation. All 15-ml screw cap falcon test tubes with conical bottom (extraction vessel) were maintained into 0.1 mol dm^{-3} HNO_3 for cleaning of any inorganic compounds and washed with doubly deionized water and then with acetone for proper sedimentation of fine droplets of the extraction solvent in the centrifuging step.

General Procedure

A 10.0 ml of aqueous solution contaning 0.2 ppm Ag and 0.01mM DDTC was placed in a 15 ml screw cap falcon test tube with conic bottom. 0.8 ml methanol, as disperser solvent, containing 60 µl carbon tetrachloride, as extraction sol-

vent, was injected rapidly into the sample solution by using a proper Hamilton syringe and the mixture was gently shaken. A cloudy solution (water/methanol/carbon tetrachloride) was formed in the test tube, Ag-DDTC complex is extracted into the fine droplets of carbon tetrachloride. The mixture was then centrifuged for 3 min at 3,500 rpm. After this process, the dispersed fine droplets of carbon tetrachloride were sedimented at the bottom of test tube (25 ± 1 µl). Finally, 10 µl of 0.05% $Pd(NO_3)_2$, as chemical modifier, followed by 10 µl of the sedimented phase were consecutively pipetted into the same auto-sampler device and the content was injected into the graphite tube and the silver content is determined by electrothermal atomic absorption spectrometry

Results

Study of the ETAAS Conditions

In order to reduce interferences and increase the accuracy, the use of a chemical modifier or a modifier mixture has become indispensable in ETAAS measurements. In the present work, we used $Pd(NO_3)_2$ as a chemical modifier. When the palladium modifier was not added, the analytical signal was gradually decreased until 50 % of the initial signal. The influence of the palladium modifier on the background level was also important. Based on the experimental results, addition of 0.05% (w/v) $Pd(NO_3)_2$ solution allowed increasing the analytical signal with considerable background reduction, without increasing the pyrolysis temperature. Because for portions larger than 10 µl the signals were not further improved, the palladium modifier injection volume was chosen as 10 µl.

The selection of an appropriate pyrolysis temperature is very important for removing as much the matrix as possible and preventing the pyrolysis loss of the analytes prior to atomization. The influence of pyrolysis temperature was studied on the absorbance, in the range of 300–1,100 °C. The maximum absorbance was achieved in the range of 300–450 °C in the presence of chemical modifier. However, when the pyrolysis temperature was over 500 °C, the signal of analyte decreased rapidly with the increase of the pyrolysis temperature. Therefore, 450 °C was selected as the optimized pyrolysis temperature for the determination of silver.

In the selected pyrolysis temperature of 450 °C, the effect of pyrolysis time on the absorbance of Ag was investigated. The results showed that the absorbance was increased when the pyrolysis time was changing from 10 to 40 s and no appreciable improvements were observed for longer times. As a result, a pyrolysis time of 40 s was chosen.

Using a pyrolysis temperature of 450 °C and pyrolysis time of 40 s, the effect of the atomization temperature, in the range of 1,000–1,500 °C, on analytical signal of Ag was also studied, The maximum signal . in the presence of chemical modifier was obtained at about 1,200 °C and remained unchanged with the further increasing of temperature up to 1,500 °C. So, the atomization temperature of 1,300 °C was selected for the further experiments. The experimental results show that atomization time has little effect on the atomic signal of Ag. Therefore, an atomization time of 3 s was selected. The unusual low pyrolysis and atomization temperatures used in this work is probably due to the fact that the components used in the dllme procedure are reducing the thermal stability of Ag.

Table I
The graphite furnace temperature program for silver determination

Step	Temperature [°C]	Ramp time [s]	Hold time [s]	Argon flow rate [ml min^{-1}]
Drying	100	1	20	250
Pyrolysis	400	5	30	250
Atomization	1,400	0	3	0
Cleaning	1,800	0	2	1,000

Effect of Type and Volume of the Extraction Solvent

Careful attention should be paid to the selection of the extraction solvent. It should have higher density rather than water, extraction capability of the interested compounds and low solubility in water. Chloroform, carbon tetrachloride and carbon disulfide were compared in the extraction of silver. A series of sample solution were studied by using 500 µl methanol containing different volumes of the extraction solvent to achieve 25 µl volume of the sedimented phase. The solubility of the extraction solvents in water is different. Therefore to recover 25 µl volume of the sedimented phase at the bottom of the test tube, it is necessary to add an excess to account for this solubility. Thereby, 75, 50 and 60 µl of chloroform, carbon disulfide and carbon tetrachloride were used, respectively.

In this experiment chloroform, carbon disulfide and carbon tetrachloride as extraction solvents obtained enrichment factors of 122.9 ± 9.4, 119.1 ± 10.5 and 127.7 ± 6.7, respectively. According to these results, variations of the enrichment factors using different extraction solvents are not statistically significantly different. Carbon tetrachloride forms a well stable cloudy solution, its sedimented phase can easily be removed by sampler to be introduced into the graphite furnace and has less consumption volume, while chloroform forms an unstable cloudy solution and carbon disulfide is difficult to be removed by sampler. Therefore, carbon tetrachloride was the best to be used.

To examine the effect of the extraction solvent volume, solutions containing different volumes of carbon tetrachloride were subjected to the same DLLME procedures. The experimental conditions were fixed and include the use of 500 µl methanol different volumes of carbon tetrachloride (40, 50, 60, 70 and 80 µl). By increasing the volume of carbon tetrachloride from 40 to 80 µl, the volume of the sedimented phase increases from 20 to 50 µl. Enrichment factor decreases with increasing the volume of carbon tetrachloride, because of the volume of the sedimented phase increases. Subsequently, at

low volume of the extraction solvent high enrichment factor was obtained. Thereby, the gain in sensitivity was achieved by using 60 µl of carbon tetrachloride.

Effect of Type and Volume of the Disperser Solvent

The main criterion for selection of the disperser solvent is its miscibility in the extraction solvent and aqueous sample. For this purpose, different solvents such as acetone, acetonitrile, methanol and ethanol were tested. A series of sample solutions were studied by using 800 µl of each disperser solvent containing 60 µl of carbon tetrachloride (extraction solvent). The enrichment factors obtained for acetonitrile, acetone, methanol and ethanol were 108.7 ± 9.1, 125.2 ± 8.8, 120.4 ± 5.3 and 115.6 ± 7.5, respectively. The results show no statistical significant differences between disperser solvents; however, the solubility of DDTC in methanol makes it a better choice.

The effect of the volume of methanol on the extraction recovery was also studied. Since, variation of the volume of methanol makes change in the volume of sedimented phase at constant volume of carbon tetrachloride (extraction solvent). Thereby, to avoid this matter and in order to achieve a constant volume of sedimented phase (25 µl) the volume of methanol and carbon tetrachloride were changed, simultaneously. The experimental conditions were fixed and include the use of different volumes of methanol 0.50, 0.8, 1.00 and 1.50 ml containing 45, 60, 75 and 100 µl of carbon tetrachloride, respectively. Under these conditions, the volume of the sedimented phase was constant (25 ± 1 µl). It is clear that by increasing the volume of methanol, the solubility of complex in water increases. Therefore, the extraction recovery decreases. Thus, 800 µl of methanol was selected as optimum volume in order to achieve better and more stable cloudy solution.

Effect of pH

The separation of metal ions by dispersive liquid–liquid microextraction involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of the sedimented phase, thus, obtaining the desired preconcentration. pH plays a unique role on metal–chelate formation and subsequent extraction. The effect of pH on the complex formation and extraction of silver from water samples was studied in the range of 1–6 by using concentrated H_2SO_4 solution(note that DDTC is a weak base). The results illustrated in Fig. 1. reveal that the absorbance is nearly constant in the pH range of 3.5–4.0. Therfore the pH 4 seems a proper choice. Moreover, to make pH 4 adjustment, the use of buffer (which are sources of contamination) is not necessary and sulfuric acid can simply be used to make the pH adjustment.

Effect of DDTC Concentration

The effect of the DDTC concentration on the absorbace was studied in the range of 0.001-1mM of DDTC. The absorbace was increased by increasing the DDTC concentration, which is well expected. It seems that slight reduction of extraction in high concentration of DDTC is due to the extraction of DDTC itself, which can easily saturate the small volume of extraction solvent. Also, at high concentration of DDTC (1mM) the background absorbance was increased. Therefore, the concentrationt of 0.01 mM DDTC was selected as the best choice to prevent any interference.

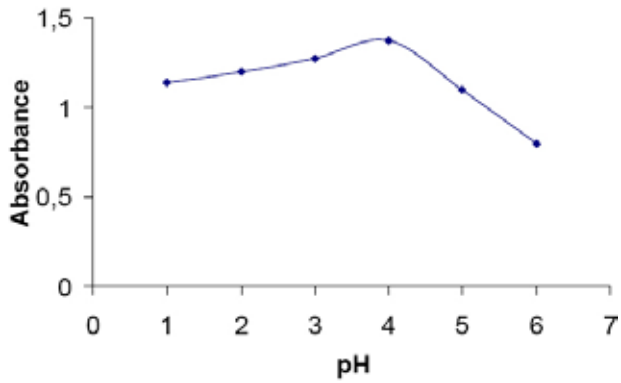

Fig. 1. **Effect of pH on silver extraction**

Effect of Salt

For investigating the influence of ionic strength on performance of DLLME, various experiments were performed by adding different amount of $NaNO_3$ (0–8 % (w/v)). Other experimental conditions were kept constant. By increasing the $NaNO_3$ from 0 to 8 %, the volume of sedimented phase increases slightly from 25 to 27 µl. The results showed that salt addition has no significant effect on the enrichment factor. It is maybe because of two opposite effects of salt addition in DLLME of silver. One of them is increasing the volume

Table II
Effect of foreign ions on the pre-concentration and determination of silver (20 ng ml^{-1})

Ion	Ion/Au ratio	Extraction recovery [%]
Li^+	10,000	103.5 ± 3.7
K^+	10,000	103.8 ± 4.8
Mg^{2+}	10,000	94.3 ± 1.9
Ca^{2+}	10,000	102.3 ± 7.3
Mn^{2+}	1,000	96.3 ± 4.7
Ni^{2+}	1,000	89.7 ± 8.7
Hg^{2+}	1,000	104.5 ± 3.8
Cu^{2+}	1,000	93 ± 1.2
Co^{2+}	1,000	101.2 ± 4.0
Cd^{2+}	1,000	93.6 ± 3.8
Pb^{2+}	1,000	95.9 ± 1.3
Bi^{3+}	1,000	94.6 ± 3.7
Cr^{3+}	1,000	94.8 ± 5.0
NO_3^-	1,000	105.8 ± 4.6
SO_4^{2-}	1,000	101.0 ± 6.2
Cl^-	10,000	103.3 ± 4.6

of sedimented phase that decreases the enrichment factor and another is salting-out effect that increases the enrichment factor. Therefore, the enrichment factor is nearly constant by increasing the amount of sodium nitrate. Therefore the experiments were performed in absence of the salt.

Effect of Coexisting Ions

The effects of common coexisting ions in natural water samples on the recovery of silver were studied. In these experiments, 10.0 ml of solutions contains 20 ng ml^{-1} of silver and various amounts of interfering ions were treated according to the recommended procedure. A given spices was considered to interfere if it resulted in a ± 5 % variation of the AAS signal. The results obtained are given in Table II.

Figures of Merit

Table. III summarizes the analytical characteristics of the optimized method, including linear range, limit of detection, reproducibility, and enhancement factor. The calibration graph was linear in the range of 6–120 ng ml^{-1} of silver. The limit of detection, defined as $C_L = 3\ S_B/m$ (where C_L, S_B and m are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively), was 1.2 ng ml^{-1}. The relative standard deviation (R.S.D.) for ten replicate measurements of 20 ng dm^{-3} Ag was 3.6 %. The enhancement factor was obtained from the slope ratio of calibration graph after and before extraction, which was about 66.

Table III
Analytical characteristics of DLLME-ETAAS for determination of Ag

Element condition	DLLME–ETAAS	ETAAS
Linear range [ng ml^{-1}]	6–120	500–20,000
Correlation coefficient (r)	0.995	0.997
Slope	2.65	0.04
Enhancement factor[a]	66	–
RSD [%], (n = 8)[b]	3.6	3.5
LOD [ng ml^{-1}][c]	1.2	80

[a]Calculated as the ratio of slope of pre-concentrated samples to that obtained without pre-concentration.
[b]At a silver concentration of 20 ng ml^{-1}.
[c]Determined as three times of the standard deviation of the blank signal, and slop of calibration curve after pre-concentration

Analysis of Natural Waters

The proposed DLLME–ETAAS methodology was applied to the determination of silver in several water samples. Tap, underground and river water were collected from the Tehran and were analyzed by DLLME combined with ETAAS for determination of silver. The concentration of silver in the tap, underground and river water samples were determined to be 17.2 ± 0.6 ng ml^{-1}, 24.7 ± 0.6 ng ml^{-1} and 30.10 ± 0.4 ng ml^{-1} respectively (Table IV). The water samples were spiked with silver standards to assess matrix effects. The relative recoveries of silver from these waters at spiking level of 10 and 20 ng ml^{-1} were 102, 98, and 100 %, respectively (Table IV). These results demonstrated that the tap, underground and river water samples matrices, in our present context, had little effect on DLLME of silver.

Table IV
Determination of Ag in real samples

Sample	Added [ng ml^{-1}]	Found [ng ml^{-1}][a]	Recovery [%]
Tap water[b]	–	17.2 ± 0.6	–
	10	27.3 ± 0.4	101
	20	37.5 ± 0.6	103
Underground water[c]	–	24.7 ± 0.5	–
	10	34.6 ± 0. 6	99
	20	44.4 ± 0.5	97
River water[d]	–	30.1 ± 0.4	–
	10	40.0 ± 0.5	99
	20	50.2 ± 0.8	101

[a]Mean of three experiments ± standard deviation.
[b]From drinking water system of Tehran, Iran.
[c]Obtained from Vardavard, Iran.
[d]From Karaj river, Iran.

Conclusions

In this paper we introduced a DLLME–ETAAS method for the analysis of ultra trace amounts of Ag in real samples such as tap water, river water and underground water. The important features of DLLME method are low cost, use of minimized toxic organic solvents, simplicity of operation, rapidity, high enrichment factor and high sensitivity and selectivity. High preconcentration factor was obtained easily through this method and a detection limit at ng ml^{-1} level was achieved with only 10.00 ml of sample. In this method sample preparation time as well as consumption of toxic organic solvents was minimized without affecting the sensitivity of the method..This method is characterized with simplicity, rapidity, reliability, safety and low cost, and is suitable for the determination of ultra-trace silver in environmental water samples.

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P02 REMOVAL OF 2-MERCAPTO-BENZOTHIAZOLE FROM SYNTHETIC WASTEWATER

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Introduction

2-Mercaptobenzothiazole (MBT) is the most important member of the benzothiazole group of heterocyclic aromatic compounds. It is a pale yellow, crystalline substance with an unpleasant odor and a bitter taste. This xenobiotic compound is used mainly in the manufacture of rubber additive chemicals but also has other uses, notably as a corrosion inhibitor, antifreeze for automobiles. It is known as a toxic and poorly biodegradable pollutant. It is able to pass conventional treatment systems, widespread with surface and underground waters and enters into organisms[1]. Data concerning biodegradation of MBT are inconclusive. Some authors[2] have suggested it is recalcitrant to biodegradation.

Genotoxicity investigations in bacterial and mammalian test systems provide some evidence indicating that MBT has the potential to induce mutations and chromosomal aberrations. Toxicity studies in rats and mice chronically exposed to MBT identified increases in various tumors[3]. Epidemiological investigations indicate that workers occupationally exposed to MBT have an increased risk of death from bladder cancer. MBT interfered with the nitrification processes and exhibited biocidal effects. MBT inhibits the degradation of easily degradable organics[4].

This work was aimed at study biodegradation of MBT and effect of this compound on activated sludge. Degradation of MBT by ozone was also studied.

Experimental

Biodegradability of MBT

Adaptation of activated sludge to MBT was carried out in semicontinuos bioreactor. We were adapted MBT with maximal concentration 25 and 50 mg dm^{-3} on the activated sludge. Time of adaptation was 3 weeks. During the adaptation we measured inhibition effect of MBT on the activated sludge by respirometric measurements.

Adsorption tests were performed both with adapted and nonadapted activated sludge to MBT. Adsorption of MBT on activated sludge was studied at various concentration values of MBT. We were measured the specific sorptive capacity of sludge at low (10, 20 and 60 mg dm^{-3}) and high concentration of MBT (100, 200 and 500 mg dm^{-3}).

Ozonation of MBT

The feasibility of utilisation of ozone for degradation of MBT was investigated in laboratory scale equipment. The experiments were performed in bubble ozonation column. The ozonation equipment consists of two glass columns, 0.04 m diameter and 1.70 m height. The first column was filled with synthetic water with MBT, and the other one was filled with solution of potassium iodide to destroy residual ozone in the outlet of the first ozonation column. The system was operated in batch mode. Synthetic wastewater containing MBT was added into ozonation reactor at the beginning of trials. Continuous flow of oxygen 30 dm^3 h^{-1} was applied for generation of ozone. The Lifetech ozone generator with the maximum ozone production 5 g h^{-1} and Lifetech ozone UV detector were used. Ozonation trials were carried out at 70 % of the power maximum of ozone generator. Initial concentration of MBT in synthetic water was 50 mg dm^{-3}.

Results

MBT is not consumed by non adapted activated sludge. Decrease of respiration rate of non adapted activated sludge was observed with the increase of 2-MBTconcentration. Rapid decrease of respiration activity was observed for activated sludge after adaptation. The endogenous respirometric rate decreased by 6 % at concentration 25 mg dm^{-3} MBT in comparison with reference model. The endogenous respirometric rate decreased by 39 % at concentration 50 mg.dm^{-3} MBT in comparison with reference model.

The decrease of dissolved MBT concentration was observed at the low concentration levels. The value of COD and TOC increased after 10 days, which is caused by increase of concentration of MBT in solution.

Table I shows the results of adsorption test carried out at lower concentration values of MBT. Negligible adsorption of MBT on activated sludge was observed at MBT concentration higher than 100 mg dm^{-3}.

According to results shown in the Fig. 1. the highest COD removal was observed during the first 20 minutes of ozonation. The initial COD value conversion achieved about 98 % after 50 minutes. The efficiency of TOC removal was 54 % after 50 minutes of ozonation. Almost total removal (96 %) of 2-MBT from the sample was achieved after 5 minutes of ozonation. Simultaneously the presence of benzothiazole was identified as ozonation intermediate. The presence of benzothiazole (BT) was identified in the sample after 50 minutes of ozonation. Fien et al.[5] show, that MBT and its breakdown products had a high affinity towards ozone as indicated by the

Table I
Specific sorptive capacity of activated sludge

c_{MBT} at solution [mg dm^{-3}]	MTB decrease by adsorption [mg dm^{-3}]	MTB decrease by adsorption [mg g^{-1}]
10	4.2	42
20	5.9	59
60	6.8	68

rates for partial oxidation and mineralization. Benzothiazole was identified as the first ozonation product, reaching up to 60 mol% of the original MBT concentration, followed by low concentration of 2(3-H) benzothiazolone.

The results of absorption of ozone in the fresh water and in the synthetic wastewater containing MBT are given in Fig. 2. The saturation concentration of ozone 23.1 mg dm^{-3} was achieved in fresh water after 35 minutes. Specific ozone consumption values were 0.78 g g^{-1} for COD and 1.88 g g^{-1} for TOC.

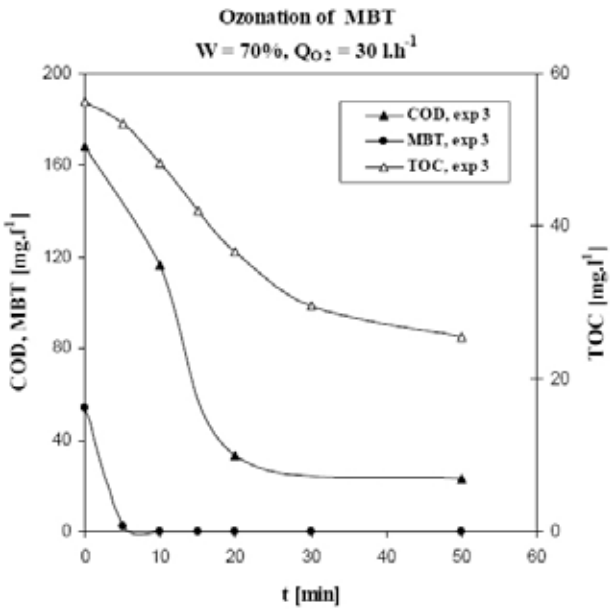

Fig. 1. **COD, TOC and MBT as a function of ozonation time at oxygen flow rate 30 dm^3 h^{-1} and W = 70 %**

Conclusions

Respirometric tests confirmed that this compound is not biodegradable. Activated sludge showed adsorption affinity to MBT at concentration values up to 60 mg dm^{-3}. Significant decrease of MBT content was observed after five minutes of ozonation. Correspondent efficiency values for COD and TOC removal were 55 and 16 %. COD removal rate 5.2 mg dm^{-3} min^{-1} was observed at 70 % of maximum ozone

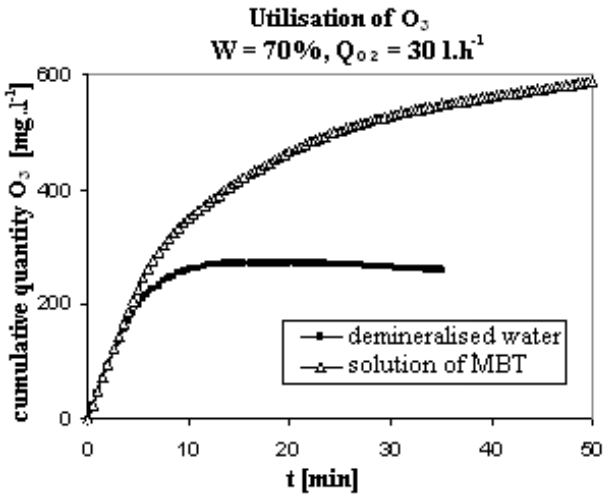

Fig. 2. **Utilization of ozone in demineralised water and in synthetic wastewater contentaining MBT**

generator power. The presence of benzothiazole was identified as ozonation intermediate. It can be concluded that MBT is readily transformed by ozonation.

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P03 HEALTH RISK ASSESSMENT BY INDOOR AIR QUALITY MONITORING

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Introduction

The risk is an inseparable part of working activity. That is why a general duty of employer is to ensure health and safety at work for all employees. They are protected by the main framework Directive 98/391/EEC, which basic principle is risk prevention, which requires risk assessment by the responsible employer.

Dangerous substances can be found in many workplaces. A recent European survey shows that 16 % of workers reported handling hazardous products and 22 % being exposed to toxic vapours[1]. As far as work accidents concern according to OSHA facts[1]:

- Every 3 and a half minutes somebody in the European Union dies from work-related causes
- Every year 142,400 people in the EU die from occupational diseases and 8,900 from work-related accidents
- Up to third of these 150,000 fatalities each year can be attributed to hazardous substances at work in the EU (including 21,000 to asbestos)

Exposure to dangerous substance occurs at any industry workplace, on farms, in vehicles, especially at chemical plants and at smaller areas, such as high school, universities, in laboratories and also at home or in office.

Dangerous substances affect human's health by different ways. Some can cause cancer, affect the reproductive function or cause mutagenic effects. Other agents may cause brain damage, be harmful to the nervous system, respiratory airways or skin.

Quality of workplace environment includes not only safety conditions at work, achieved by continual risk assessment and elimination of these risks, but also means a clean and healthy environment. The working environment is formed by different microclimatic conditions, such as level of noise, lighting, quality of indoor air, surrounding temperature, level of humidity etc. Each of these factors has a great influence on worker's health and productivity of his work.

Since many people spend a large part of their lives in closed areas – in an office, at school, in transport – clean air becomes essential for good health and this is especially true when speaking about indoor air.

Posing the Problem

As it was mentioned above among different workplaces, school's environment can be found. Nowadays school area consists of not only usual class-rooms, but also covers wide range of specialized working facilities, for example chemical laboratories.

In this article we will focus on chemical hazards in the laboratories. People who work or study in a chemical laboratory are exposed to many kinds of hazards, e.g. chemical substances, mechanical hazards, biological agents, physical factors, psychological conditions and so on.

Special feature of such work environment is that level of chemical threats rises steeply, since the quantity and range of chemicals are higher than in any other place of usual life. Many agents are highly flammable and explosive, their careless handling and storage may result in fire ignition and explosions. Toxic gases, fumes and liquids may be produced and cause poisoning or infection of personnel and students. Some chemical agents have carcinogenic or mutagenic properties.

Indoor air quality is also one of the important factors in the working space and shouldn't be underestimated. The influence of air quality which we breathe can be extended on two main problems: long-term health affection and work-related accidents. The first problem is connected with long-term exposure of workers and students to low concentrations of different substances in the air (the most followed are carbon and nitrogen oxides, radon and Volatile Organic Compounds – VOCs). Usually it can be solved by periodical measuring of concentration, assessing the possible risks of this exposure and taking correcting measures, for example improving ventilation, changing working regime, and using proper personal protective equipment.

The second problem concerns working-related accidents. On the one hand any laboratory worker or student can be exposed to toxic gases and fumes, which may unexpectedly escape from their container or come out from the by-side effect of reaction. These substances in the air can cause different effects:

- Acute poisoning or injury of the organism
- Cause allergies
- Irritate eyes or breathing system

Usually there are few people in laboratory at the same time. If one of them will lose self-control under certain conditions, such as bad vision, suffocation, pain, his behavior becomes dangerous for others and can lead to emerging other hazards for surrounding people.

Objects of Research

According to EC Directive 1999/13/EC (Solvent Emissions Directive), Volatile Organic Compounds (VOCs) are functionally defined as organic compounds having at 293.15 K (i.e., 20 °C) a vapor pressure of 0.01 kPa or more, or having a corresponding volatility under particular conditions of use.

In a majority solvents need to be managed carefully due to their volatility and general flammability, in particular during loading and unloading, storage and when using large quantities.

Commonly, producers enclose Safety Data Sheets to their products with necessary using, handling and storage recommendations:

- Safe exposure limits and techniques for managing flammability
- Information on the main hazards, how to protect against them and the steps to take in an emergency
- Occupational exposure limits (OELs)
- Handling, storage, transport, spills and disposal advice
- Regulatory information such as classification and labeling
- Toxicity and environmental information

In chemical laboratory among other used chemicals one of the most famous are organic solvents, which usually referred to as group of Volatile Organic Compounds (VOCs). VOCs are compounds given off by a number of other indoor sources. Concentrations of most volatile organic compounds is higher in indoor than outdoor air. They commonly can be found in household, institutional, and industrial cleaning and maintenance products, and in building and finishing materials. Other sources of VOCs include the burning of fuels such as gas, wood and kerosene, as well as tobacco products. VOCs can also come from personal care products (perfume and hair spray), cleaning agents, dry cleaning fluid, paints, lacquers, varnishes, hobby supplies, and from copying and printing machines[2].

In enclosed spaces, VOCs can cause eye, nose, and throat irritation, dizziness, headache, memory and visual impairment. Higher concentrations may cause irritation of the lungs, as well as damage to the liver, kidney, or central nervous system. Some VOCs are suspected to cause cancer in humans and have been shown to cause cancer in animals. The health effects caused by VOCs depend on the level and length of exposure.

The real concentrations of VOCs are usually orders of magnitude below the occupational threshold limit values (TLVs). However, some VOCs may be present above their human odor thresholds (OTs). Beside odor annoyance, VOCs at sub-TLV level may cause non-specific health effects such as eye and upper respiratory airway irritation, headache and increased weariness[3].

The main route of exposure to solvents is via inhalation. Occupational exposure limits (OELs) set the airborne concentration of a substance that workers can be exposed to, day after day without any adverse health effects. OELs are normally set for an 8 hour day^{-1} and a 40 hour $week^{-1}$ and are continuously reviewed by national and EU authorities. OELs for the majority of hydrocarbon and oxygenated solvents are set between 10 and 1,000 parts per million depending on the volatility and toxicity of an individual substance[4].

According to what was stated above as objects of research were chosen some organic solvents which are frequently used in chemical laboratories of the Faculty of Chemistry, Brno University of Technology. Acquired data will be used for the following health risk assessment in chosen workplaces.

Managing the Problem

A four-step approach to risk assessment exists[4].

- Make an inventory of the substances used in the processes in the workplace and those generated by the process such as welding fumes or wood dust.
- Collect information about these substances, i.e. the harm they can do and how this can happen. Safety data sheets (SDS), which must be provided by the supplier of a chemical, are an important source of information.
- Assess exposure to the identified dangerous substances, looking at the type, intensity, length, frequency and occurrence of exposure to workers, including combined effects of dangerous substances used together and the related risk.
- Rank the severity of the established risks.

As we can see from this list of steps, assess exposure is one of the important parts. Exactly, this direction was chosen to exposure prevent and control of the dangerous substances in chemical laboratories.

The purpose of this study is to measure the concentration of VOCs, which are used in chosen chemical laboratories and to assess possible occupational health risks. The aim is to protect people who are supervising the practice work and working in the harmful environment for a long time (occasionally more than 8 hours). This research work is supposed to be conducted for a long-term period.

The measurements will be taken in different working areas (for example laboratory of organic chemistry, storage area). For conducting the analysis a passive air sampling by Radiello cartridges with charcoal is chosen with followed CS_2 desorption and GC with FID or GC-MS analysis.

Method used to sample collection and analysis, passive sampling (by means of new trade product Radiello®), is considered to be a simple and rather cost-effective. Unlike active sampling, passive samplers require no expensive pumps and are simple to use (no calibration is needed). Other advantages are:

- Compact, portable
- Offer indication of average pollution levels over time periods of 8 hour to weeks/months
- Relatively low-cost in compare with other methods
- Applicable for personal monitoring and indoor air analysis

Conclusions

Important point of view for assessing acceptable working place is its safety requirements and conditions which will surround the employees, what means working environment, which consists of physical, chemical and other factors. These factors can unfavorable affect worker's health by their poor quality or quantity, as well as duration of this affection.

Everybody wants to work in the safety and high-quality working environment. This statement enclosed in the European legislation and imposes some obligations on employers:

- Assess the possible risks
- Take measures to eliminate or reduce the risks
- Effectiveness control of the preventive measures and their review

A healthy work environment means the monitoring and maintenance of hygienic limits in the workplace, such as temperature, humidity of air, proper chemical composition, intensity of working process etc.

University area with its laboratories is a special working environment. Students and other workers, who is working or studying in such laboratories, are exposed to many kinds of hazards. But the main difference is that laboratory work involves a greater variety of possible chemical hazards. There are many agents, which have flammable, explosive, toxic and other characteristics.

Besides obvious risks, which can emerge during laboratory work, there are also non-evident ones, for example, air quality.

Among different chemical agents, there is a group of the most used, such as organic solvents. Some of them could be very harmful for human's health, but usually for assessing their affection it'simportant take into account their features, time of exposure and concentration during exposure.

For this purpose the methodology of indoor air analysis is going to be developed, which is supposed to help in assessing possible occupational health risks, posed by quality of indoor air in chemical laboratories. The indoor air analysis is conducting by passive sampling with following GC with FID or GC-MS analysis on long-term conditions.

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P04 NITROGEN IN BREEDING LAYING HENS AND ENVIRONMENT PROTECTION

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Introduction

At EU–15 level the gross nitrogen balance in 2000 was calculated to be 55 kg ha^{-1}, which is 16 % lower than the balance estimate in 1990, which was 66 kg ha^{-1}. In 2000 the gross nitrogen balance ranged from 37 kg ha^{-1} (Italy) to 226 kg ha^{-1} (the Netherlands). All national gross nitrogen balances show a decline in estimates of the gross nitrogen balance (kg ha^{-1}) between 1990 and 2000, apart from Ireland (22% increase) and Spain (47% increase). The following Member States showed organic fertilizer application rates greater than the threshold of 170 kg ha^{-1} specified by the Nitrates Directive in 2000: the Netherlands (206 kg ha^{-1}) and Belgium (204 kg ha^{-1}). The general decline in nitrogen balance surpluses is due to a small decrease in nitrogen input rates (–1.0 %) and a significant increase in nitrogen output rates (10 %). The gross nutrient balance for nitrogen provides an indication of potential water pollution and identifies those agricultural areas and systems with very high nitrogen loadings. As the indicator integrates the most important agricultural parameters with regard to potential nitrogen surplus it is currently the best available approximation of agricultural pressures on water quality. High nutrient balances exert pressures on the environment in terms of an increased risk of leaching of nitrates to groundwater. The application of mineral and organic fertilizers can also lead to emissions to the atmosphere in the form of nitrous dioxide and ammonia, respectively. Gross nitrogen balances are above 100 kg per ha in the Netherlands, Belgium, Luxembourg and Germany. They are particularly low in most Mediterranean countries given the overall lower livestock production in this part of Europe. It is currently not possible to provide gross nitrogen balance estimates for the new EU Member States and the accession as the relevant statistical data are under elaboration. National balances, however, can mask important regional differences in the gross nutrient balance that determine actual nitrogen leaching risk at regional or local level. Individual Member States can thus have acceptable gross nitrogen balances at national level but still experience significant nitrogen leaching in certain regions, for example in areas with high livestock concentrations. There are a number of regions where pig livestock units have increased by more than 25 % between 1990 and 2000 (for example, north-western Denmark, north-western France, north-eastern Spain and northern Belgium). These are likely to be regional 'hotspots' for high gross nitrogen balances that can lead to environmental pressures. Member States with high nitrogen balances are making efforts to reduce these pressures on the environment. These build on a range of different policy instruments, requiring considerable political effort to succeed given the significant social and economic consequences of reducing livestock production in many affected areas[3,4].

Experimental

We realized experiments with laying hens *Shaver Starcross* 288, which ingested feed mixture with different protein content. In six experiments laying hens fed feed mixture with protein contents 173.10 g per kilogram and in four experiments were used feed mixture with protein contents 146.12 or 146.68 g per kilogram. It is soya cereal type assigned for laying hens. The laying hens ingested fodder *ad libitum*.

Within experiments were researched:

- protein contents in feed mixture in one kilogram (chemical analysis – Kjeldahl method and calculation),
- excreted nitrogen [g kg^{-1}] in dropping per bird and day (chemical analysis – Kjeldahl method and calculation).

Results

Higher protein contents 173.10 g per kilogram of feed mixture resulted in excreted dropping at laying hens higher nitrogen contents in compare with protein contents 146.40 g per kilogram feed mixture. After ingestion of feed mixture with protein content 173.10 g per kilogram was nitrogen content in dropping from 1.88 g per bird and day. The laying hens, which fed feed mixture with protein contents 146.40 g per kilogram, excreted the nitrogen in dropping 1.38 g per bird and day. From these results follows that near decrease protein contents in feed mixture from 173.10 to 146.40 g per kilogram is possibility of nitrogen decrease in excreted dropping at laying hens about 26.60 % per bird and day. This different of decrease of excreted nitrogen in dropping at layers is statistically significant ($P<0.001$). Correlation coefficient between content of crude protein in feed mixture and content of nitrogen in dropping at layers has high level $r = 0.99$.

Low-protein diet system for layers with addition of amino acids is beside biologically-cattle-breeding, economical and ecological too.

Progressive decrease of proteins content to 146.0 g per 1 kg feeding mixture set up the order of limiting amino acids for layers: methionine, lysine, tryptophane and threonine[5].

On bases of results model trials on layers were concluded that feeding, fat-enriched mixture supplied DL-methionine, choline chloride and vitamin B_2 by need of the effective

Table I
Excreted nitrogen [g kg^{-1}] in dropping at laying hens, which ingestion of feed mixture with different protein contents

Trial	Index		SD	$v_{\%}$	t-test
1st	CP [g kg^{-1}]	173.10	0.91	0.52	55.68^{+++}
2nd	CP [g kg^{-1}]	146.40	0.32	2.68	
1st	N [bird day^{-1}]	1.88	0.005	0.22	18.52^{+++}
2nd	N [bird day^{-1}]	1.38	0.02	1.57	

substance possible reach adequate the laying, egg weight and their quality on the economical using of the feedstuff and lower environment load[1]. In order experiment autors confirmed results of decrease of excreted nitrogen in dropping at laying hens[2].

Conclusions

The research results about nitrogen in breeding of laying hens in relationship to environment protection confirmed the possibility its decrease. One of the possibilities is decrease of protein contents in feed mixture. The decrease of crude protein content in feed mixture from 173.10 into 146.40 g per kilogram (about 15.43 %) is possibility of decrease of excreted nitrogen in dropping at laying hens about 26.60 %, which is statistically significant ($P < 0.001$). Correlation dependency between content of nitrogen in dropping at laying hens and content of crude protein is high, $r = 0.99$.

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P05 TESTING OF VARIOUS SORBENTS FOR COPPER REMOVAL FROM ACID MINE DRAINAGE

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Introduction

The elimination of the consequences of mining activities belongs to the most serious environmental problems nowadays. Acid mine drainage (AMD) with high metal concentrations and usually with low value of the pH (about 2–4) is mainly a result of chemical oxidation of sulphides and other chemical processes in overflowed mines, mining waste dumps and tailings. This water may transfer various heavy metals in a dissolved form, such as Fe, Cu, Al.

The abandoned Smolník mine is regarded as an environmental loading in the Central Europe region, where AMD is generated and discharged from abandoned mine and contaminates the Smolník Creek catchment. This acid mine drainage (AMD) with pH 3–4 contents high metal concentrations that vary in dependence on rainfall intensity (e.g. Fe 500–400 mg dm^{-3}; Cu 3–1 mg dm^{-3}; Zn 13–8 mg dm^{-3} and Al 110–70 mg dm^{-3}) (ref.[1]).

There are various physical-chemical methods of treatment such polluted water e.g. neutralisation, ion exchange, precipitation, sorption, membrane processes, filtration. The choice of the suitable methods is based not only on concentration of heavy metals in surface water but also on economical factors.

Sorption belongs to effective and economically acceptable methods for heavy metals removal. Deorkar and Tavlarides[1] developed an adsorption process of inorganic chemically active adsorbents (ICAAs) to selectively recover Fe^{3+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+} from AMD solutions without neutralization. More than 75 % of copper was removed from solutions by active carbon and biosorbents prepared from mosses, the highest sorption capacity had active carbon. On the other hand, pH values in the presence of active carbon increased almost to 9, thus copper was precipitated from the solution[3].

The paper deals with utilization of four types of sorbents (zeolite, active carbon and an atypical sorbents usually used for oil pollutants removal from surface water: turf brush PEATSORB – a hydrophobic material and universal crushed sorbent ECO-DRY) for cooper removal from AMD Smolník and presents influence of them on Cu decreasing under various conditions. The change of pH has been monitored, too.

Experimental

For study of Cu ions removal from acid mine drainage by adsorption, zeolites (granularity 0.5–1 mm, 2.5–5 mm, 4–8 mm) (Zeocem, a.s., Bystré, Slovakia), active carbon (granularity ≤0.1 mm), turf brush PEATSORB and universal crushed sorbent ECO-DRY (REO AMOS Slovakia) were used.

Because the experiments were carried out using untreated AMD (shaft Pech, locality Smolník, Slovakia), which is very unstable, new sampling of AMD for every experiment was realised. Copper removal efficiency by sorptive materials was tested at laboratory temperature under static conditions. 5 g zeolites, 1 g active coal, 5 g turf brush PEATSORB and 5 g universal crushed sorbent ECO-DRY, were overflowed with 100 ml of raw AMD for 24 h, then the mixtures were filtrated.

The dependence of Cu concentration decreasing on time (1; 3; 5; 10 min) was investigated under dynamic conditions using turf brush PEATSORB. In filtrate was determined pH (METTLER TOLEDO) and Cu by Bicinchoninate method (Colorimeter DR 890, HACH LANGE). Test of Cu precipitation in AMD was carried out by raw AMD samples of 100 ml, each were titrated to pH end points ranging from 4 to 8 using NaOH (0.5 mol dm^{-3}). During titration, the AMD solution was continuously stirred and the pH was monitored. When the preset pH end point was reached, the titrated solution was filtered to remove precipitated metals. The filtrate was used for characterizaton of copper solubility as a function of pH.

Results and Discussion

The untreated AMD (pH 3.72, Cu 2.22 mg dm^{-3}) was taken for adsorption efficiency determination of various sorbents. In Table I efficiencies of used sorbents on Cu removal from AMD are presented. In case of zeolite (2.5–5.0 mm), there was irregularity obserwed because the best adsorption effect was expected in the finest one. This fact can be explained as the structure faillure of the finest zeolite by acidity of environment. Turf brush PEATSORB was the most efficient (54.5 %) from tested sorbents.

Table I
Efficiency of various sorbents on Cu removal

adsorbent	pH	Cu [mg dm^{-3}]	efficiency [%]
zeolite (0.5–1.0 mm)	3.5	1.94	12.6
zeolite (2.5–5.0 mm)	3.7	1.79	19.4
zeolite (2.5–5.0 mm)	3.6	1.93	13.1
active coal	4.3	1.98	10.8
ECO DRY	3.7	2.17	2.2
PEATSORB	3.1	1.01	54.5

All used sorbents resulted in slightly pH decrease (expting active coal) that was positive fact because as it is seen in Fig. 1. pH above 4 is connected with precipitation of copper (for AMD pH 3.92, Cu 1.38 mg dm^{-3}).

As Fig. 2. shows 55.8 % of copper were removed after 3 minutes and the longer time of stirring wasn't efficient. The pH decreasing can be explained as ion exange process by humic acid in turf brush[4].

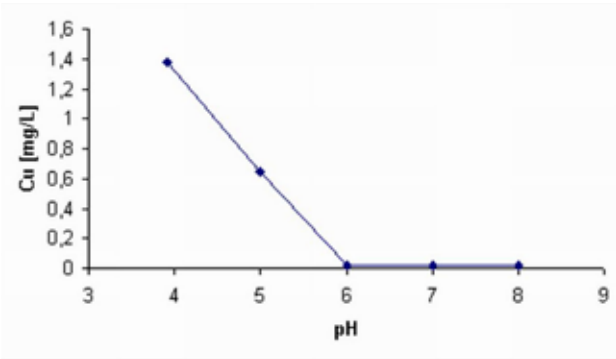

Fig. 1. **Influence of pH on Cu precipitation from AMD**

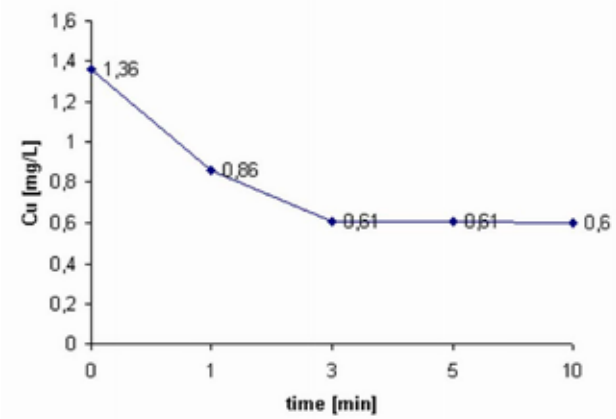

Fig. 2. **Dependence of Cu removal from AMD versus adsorption time**

Conclusions

This study shows possibility of the natural adsorbents utilisation for Cu removal from acid mine drainage. Turf brush PEATSORB was the most efficient for copper removal – decreasing of Cu concentration in AMD was about 54.5 % under static conditions and 55.8 % in stirred sample during 3 minutes. Based on experimental results we can state that chosen adsorbents haven't influenced pH increasing above 4 excepting active coal hence the Cu removal can be the result of adsorption proces.

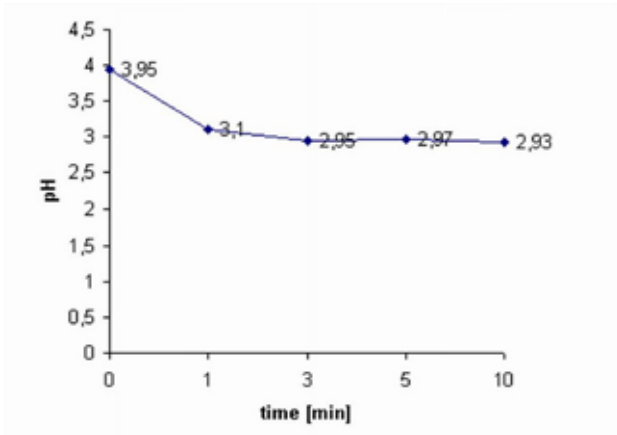

Fig. 3. **Dependence of pH change during Cu removal from AMD by PEATSORB**

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P06 INFLUENCE OF THE COMPOSITE SORBENT ON THE CONTENT OF SELECTED ELEMENTS IN THE SEDIMENT LOAD OF THE WATER RESERVOIR AND SLUDGE BED

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Introduction

Sediment load of the streams and water reservoirs (hereinafter as WR) are a result of erosive and sedimentation processes of the respective basins. The term "sediment load" is used in hydrology according to the norm actually in force[1]. Geology and geochemistry[2] use the terms "river and bottom sediments". The mining waste deposited in mine sludge beds[3] constitute a residue of mining and treatment processes. Pollution of WR with sediment load causes problems in the decreasing of water content in the WR[4] aggravating the protection of the surrounding territory against floods, etc. That is why the sediment load (bottom sediments) need to be removed from the WR[5]. Due to great amounts of sediments it needs to be decided not only on how to extract the sediments, but also on how to store or dispose of them.

Sludge beds are objects where waste created at mining extraction of raw materials and mainly created as a result of the following treatment technologies is deposited or sedimented. They are designed and built so that they do not constitute a danger for the surrounding environment. Despite the facts mentioned above the unwilling seepage of waters from the sludge beds to the ground water occurs. The waters from the sludge bed draining containing various elements flow into the surface streams. Depending on their level of contamination by various elements, mainly heavy metals (HM) the water in the surface streams and then their sediments are contaminated[6,7].

The sediment load of streams and reservoirs may be classified as hydromorphic, subhydric soils[8]. However, there is a difference resting in the method of their contamination by various elements. They are situated in a different aquatic environment and catch contaminants from several sources. They are contaminated by a wider spectrum of elements than soils what makes their use[9] or treatment more complicated. That is fully true also about the sediment load and the mining waster in the basins of the Hornád River (Fig. 1.) treatment of which is using a composite sorbent is the subject of this paper (poster).

The literature proves that only the treatment of drinking waters[10], industrial waters[10,11] and soils using natural zeolites was successful. There are published results of an experimental testing of other natural materials, but they relate to soil treatment[12,13] only. However, it seems that there is a need to find a specific technological procedure with a specific sorbent[14]

Fig. 1. **Situation map of the Hornad river basin**

for the treatment of soil, sediment or mining waste deposited on the dumping sites (dumps) or sludge beds (Fig. 2.) for a longer period of time.

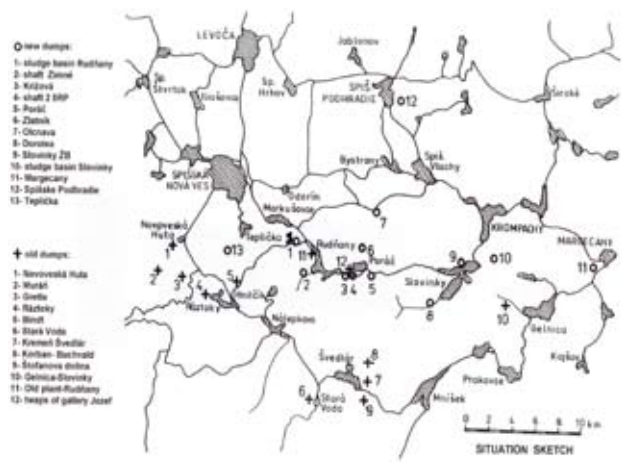

Fig. 2. **Situation map of the dumps localities in Hornad river basin**

Experimental Works

The most suitable solution of the problem of HM contaminated sediments should be their extraction after previous solution of reduction of the content or stabilization of dangerous HM contained in the sediment load (bottom sediments). That is why the first laboratory experiments were carried out at the beginning of 2007 monitoring the impact of the inorganic composite sorbent to reduce the content or stabilization of the elements in the sediment load of the water reservoir "Ružín I". According to the producer of this sorbent it is made from natural raw materials. It is mixture of atomically clays, smectite basalt tuffs, alginate, dolomite, gypsum, zeolite, coal and others. Proportion of individual components is trade secret. Qualitative mineralogical analysis of this sorbent was made by X-ray diffraction analysis. It contain amorphous phase, calcite (>15 %) as dominant minerals and quartz, sericite, chlorit-caolinite, dolomite (3–15 %)

as accompanying minerals. The impact of the mentioned sorbent on the reduction of the HN content in the sediment load was observed in laboratory conditions in compliance with the producer's recommendation in form of a 120 and 333 days experiment

The procedure of the 120-day experiment was as follows. First, 9.5 g of the sediment were weighted and 0.5 g of the sorbent added corresponding to 5 per cent by weight. The samples were put in 250 ml PVC bottles for 120 days and poured with 10 ml of the destilled water. After expiration of 120 days to each of the samples a 100 ml of 2M HNO_3 were added as a leaching agent. Then, the samples were shaken for 6 hours and sedimented for 1 hour.

A similar procedure was carried out in connection with the 333 days experiment. First 9.5 g of the sediment were weighted and 0.5 g of the mentioned sorbent added corresponding to 5 per cent by weight in accordance with producer´s recommendation. The samples were put in 250 ml PVC bottles for 333 days and poured with 10 ml of the destilled water. After expiration of 333 days to each of the samples a 100 ml of 2M HNO_3 were added as a leaching agent. Then, the samples were shaken for 6 hours and sedimented for 1 hour.

In both cases the contents of selected elements Cu, Cr, Ni, Pb and Zn were ascertained using AAS methods. For determination of the content of Cu and Zn flame atomic absorption spectrometry and for Cr, Ni and Pb graphite tube atomizer were used.

Results and Discussion

The results of the experiment when the composite sorbent affected the samples of sediment load from water reservoir for 120 and 333 days are contained in Table I.

The results of the experiment when the composite sorbent affected the samples of mining waste from sludge bed for 120 and 333 days are contained in Table II.

The content of elements of Ni, Cr and Pb in the samples marked like 1C–4C as well as in the samples containing the composite sorbent (CS) is under the lowest limit TV, norm for environment[15] and that is why the results are not in Table I and are not commented. The remaining samples marked like 120 d CS have shown the decrease of Cu from 4.53 to 8.20 % and from 11.55 to 38 % for Zn after 120 days. After 333 days the decrease of the content of Cu was ascertained from 5.55 to 15.83 % and Zn showed the content decrease from 11.26 to 37.1 %.

The cont ent of Ni and Cr in procured control samples (C) as well as in samples containing the composite sorbent (CS) was under the lowest limit TV, of the norm for environment15 and that is why the results are not commented.

The remaining samples marked as CS have shown the decrease of Cu from 7.25 to 11.65 %, Pb from 7.10 to 13.81 % and Zn from 15.36 to 19.68 % after 120 days. After 333 days the decrease of the content of Cu was ascertained from 11.23 to 14.76 %, Pb from 5.87 to 11.57 % and the content of Zn decreased from 7.67 to 9.81 %.

Table I
Influence of composite sorbent on content of heavy metals of sediment load from the water reservoir

Sample	Cu	Zn
	[mg kg^{-1}]	
1 C	151	76.4
120 d CS	140.5 (7 %)	47.35 (38 %)
333 d CS	127.1 (15.83 %)	48.05 (37.1 %)
max. % of decrease	15.83	38
2 C	168	386.8
120 d CS	157 (6.55 %)	306.6 (20.57 %)
333 d CS	142.9 (14.94 %)	311.7 (19.25 %)
max. % of decrease	14.94	20.57
3 C	48.8	54.9
120 d CS	44.8 (8.20 %)	36.3 (33.88 %)
333 d CS	44.0 (9.84 %)	38.8 (29.33 %)
max. % of decrease	9.84	33.88
4 C	342	310.8
120 d CS	326.5 (4.53 %)	274.9 (11.55 %)
333 d CS	323 (5.55 %)	275.8 (11.26 %)
max. % of decrease	5.55	11.55

1C–4C control sample of sediment load without composite sorbent;
120 d CS a 333 d CS – samples of sediment load with composite sorbent after 120 and 333 days

Table II
Influence of the composite sorbent on the content of heavy metals in the waste from the mining sludge bed

Sample	Cu	Pb	Zn
		[mg kg^{-1}]	
Dam C	103	1,296	1,382
120 d CS	91 (11.65 %)	1,117 (13.81 %)	1,110 (19.68 %)
333 d CS	87.8 (14.76 %)	1,146 (11.57 %)	1,276 (7.67 %)
max. % of dec.	14.76	13.81	19.68
Lagoon C	552	2,351	2,364
120 d CS	512 (7.25 %)	2,184 (7.10 %)	2,001 (15.36 %)
333 d CS	490 (11.23 %)	2,213 (5.87 %)	2,132 (9.81 %)
max. % of dec.	11.23	7.10	15.36

C – control sample from the mining sludge bed without composite sorbent
120 d CS a 333 d CS – samples with composite sorbent

Application of the composite sorbent also decreased the contents of Cu, Pb and Zn in both samples but the percentage of the content of HM is insignificant. Due to very significant exces of the content of Cu, Pb and Zn compared to the highest limit – IV, of the norm for environment[15]. Recycling of the waste deposited in the sludge bed should be considered

as more favorable aiming at gaining Cu, Pb and Zn. It has been the very first experiment and so our opinion may not be considered as a decisive one.

Conclusions

- When verifying the content of the composite sorbent on the content of heavy metals in the WR sediment load and wastes in the sludge bed, the decrease of the content of Cu, Pb and Zn has been ascertained. The decrease is not significant. NO other sorbents were used for the experiment and so composite sorbent cannot be recommended for the HM decreases.
- A long-term verification is needed for the recommendation of the application of used sorbent to decrease the content of HM in sediment load as well as the mining waste. The results need to be considered as preliminary and they require further verification in the conditions "in situ" for the minimum of 2 years.
- Further experiments should focus on the comparison of the used sorbent with sorbents that are accessible and experimentally tested, such as zeolites, bentonites, kaolinites and based on experimental results the most suitable sorbents should be proposed for the sediment load, wastes from sludge beds or soil.
- Today, the verification of the respective sorbent is underway in various foreign institutes. It would be useful to study the results, if they are published. Later after further experimental verification it using in our condition can be recommended.

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P08 ENGLISH FRO CHEMISTS CAN BE PHUN

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"To teach or not to teach any special English tailored to serve a particular profession which in our case means English for chemists? ...Isn't deep, substantial knowledge of general English everything one needs to be able to communicate?"

Such questions are often ask by many a technically oriented colleague I meet at my workplace. To answer them, I always remember Elaine Horowitz, PhD from the School of Education of UT at Austin (TX). She openes her doctoral classes on Foreign Language Acquisition with the following definition of language competence:

"Foreign language competence can be defined as the ability of authentic self presentation in that language. In other words, you can be called competent in L2 if the level of your education is reflected in the way you use this language. That means if a native speaker finds out who you are from the way you communicate."

Accepting this approach to competency we can say with confidence that English for specific purposes has its irreplaceable position in a postsecondary curriculum.

ESP may not always focus on the language of one specific discipline or occupation, but it is supposed to introduces students to common features of academic discourse in the sciences or humanities, frequently called English for Academic Purposes (EAP),

I would like to communicate some ideas and experience of teaching ESP classes at the Faculty of Chemistry of BUT. Our mission of teaching English for future chemists is more or less challenged by these phenomena:

(i) Absence of appropriate teaching materials on the market. We have not found any material in bookstores which would fit our specific needs and could be used as an English textbook for the chemistry students. (This said with no intention to blame any bookstore, of course!)

(ii) The different language experience of the students who come to our school. Their language proficiency often ranges from the true novice level to the advanced. The beginners and lower intermediate students have the possibility to attend two semesters of general English classes before they register for ESP. However, the different level of their language knowledge in the ESP classes cannot be fully eliminated as you could hardly expect them to make the leap from the beginner level to the upper intermediate or even advanced one in one year.

(iii) The absolute majority of our students strongly oppose and almost detest memory based learning as they are used almost entirely to rely on their ability of logical reasoning. ("We would not have been here at BUT if we had been able to memorize. If we had been able to memorize, we would sure have studied law or humanities!)

(iv) Students, especially in the previous years, had often a feeling that English was not their major specialization. That they did not come here to study English but chemistry. Despite the gradual change in this approach, there are always some who enter to the English classroom saying: "We are so tired from the previous instructions ...," The laboratory classes we have just had were so tedious...", We are just after organic chemistry/physics, math, … classes and tests, absolutely drained both intellectually and physically, please, do not want us to talk…" etc, etc.

(v) Last but not least challenge is that the technically oriented students are not such good "natural speakers" even in their mother language as the students of the humanities. ("My goodness gracious, I do not know what to say even in Czech. And now you want me to communicate it in English on top of it…!")

What have we done to cope with the above mentioned challenges?

Ad *(i)* The first step to overcome the gap in the teaching materials on the market was the creation and implementation of the teaching material of our own. At the earlier stages of our professional lives at our school we always prepared handouts and distributed them at the beginnings of the lessons. The dramatic change in our work occurred when the internet was installed into most of our classrooms. The availability of this medium made a great stimulus for us to create an internet based textbook which we called English for Chemists www.fch.vutbr.cz/ang2. When creating this material we had the following objectives in minds:

The structure of our faculty – it is reflected in the selection of the topics as the subject matter of the individual lessons corresponds with the specialization of our institutes...

Proportional balancing of the lesson content so that all four major skills could be developed equally. The use of 4 different icons (indicating writing, talking, listening and reading) to label the individual exercises gives us a quick orientation.

We tried hard to bring sound into the reading activities. – Why do we emphasize sound so much? Everybody will agree that priority number 1 in foreign language instructions is to reach fluency. Fluency can be defined as the ability to understand and speak instantly, e.g. without translating. Fluency enables us to talk easily with native speakers. They easily understand us and we easily understand them The only way how to reach it goes through listening. That means we shall not get fluency in English just by reading English articles or learning grammar rules. To become fluent, students must have a lot of understandable, repetitive listening. It means, they will not learn English only with their eyes, but they must learn English with their ears. It is important to know that powerful listening must be repetitive and understandable (A. J. Hoge). We managed to answer this demand by the following ways:

- the reading sections of the textbook have been vocalized by a native lecturer

- each vocabulary section is competed with a hyperlink going to Merriam-Webster on-line dictionary which is soundtracked
- each lesson is completed with a substantial number of hyperlinks going to various sound tracked specific articles, animations, video recordings, demonstrations etc. that can be utilized for this purpose.

Exposing students to the culturally authentic sources as much as possible. (e.g. videotapes, radio and TV broadcasts, films, songs etc.) – this material has long been advocated by foreign language educators as stimulating pedagogical aids. One of the best sources of the authentic materials for classroom instructions is the Internet. Target language sites accessed through the Internet offer both teachers and students a wealth of authentic materials. The advantage of such materials is that they are current and readily available. Their topicality can be easily maintained by the regular visits of these web sites. That is why we completed the individual lessons with hyperlinks going to various specific video sections, songs, soundtracked animations, classroom instructions etc.

However, it is important to remember, that the documents found on the Web, like all authentic materials, have been created by and for native speakers of the language. That means they are not written with the language learning in mind. For this purpose we assessed these documents from the perspective of their general understandability first. Then we have completed these materials with the tasks that suit even the less proficient students.

Ad *(ii)* The different language experience of our students

We tried to meet the needs of the less proficient students by creating each textbook lesson in two versions. Firstly it is a printable version which the student will print and bring with him/ her to the class. Its format leaves plenty of space for writing down student's own answers. Secondly, each unit has also the version completed with the clues to all exercises. This serves mainly to the less advanced ones to go through the explain subject matter at home again and check their answers or possibly to look up the correct answers.

Besides, each lesson is ended with a short self test enabling the students to evaluate their comprehension.

Ad *(iii)* Unwillingness to learn vocabulary and idioms by memorizing

Experts say that most of people must hear a new word 30 times to remember it forever. To know a word and instantly understand it, you probably need to hear it 50–100 times. The students must know that it is not enough to listen to a new word just once or twice. As the time allocation of our classes do not allow to practice repetitive listening to much at school, the students are strongly recommended to go to all the listening material again at their leisure, e.g. at home, and repeat all the listening activities as many times as possible.

Ad *(iv)* How to engage the students who are tired from the previous "more important" classes? The answer is: make the English for chemists fun.

Here the Internet makes a great aid again. We can use various science/chemistry oriented humor, interactive quizzes, crossword puzzles and various songs created by native students and teachers with the aim to bring fun and entertainment into their chemistry classes. Songs make excellent memory boosters. AS most people have strong musical memory, we take can advantage of this fact to make memorization and learning easier. Putting words to music instantly makes those words more memorable.

Besides memorizing song verses our learners can profit from retelling the jokes, playing memory boosting quizzes (e.g. flash cards), solving crosswords etc.

This can be effectively completed with the popular game based on the repetition of the words. Everybody who has ever visited a language school will sure the remember the game called: In our local super market we can buy/I will pack into my suitcase … Its variations on the premises of our school sound: In our chemistry laboratory we have … (the names of various laboratory equipment follow) .../Breathing carbon monoxide will cause… (Symptoms of CO poisoning)/Iodine deficiency causes… etc, etc.

Ad *(v)* Shyness to speak

During a semester students must prepare their own mini-presentations to meet one of the necessary conditions of getting a credit. Prior this activity they are provided with all the necessary phrases and expressions so that they learn how to address the audience, introduce themselves, express the purpose of the paper/presentation, signpost the presentation, move on in the course of it, describe the pictures, verbalize graphical data, ask checking-up questions, invite questions, finish the presentation… Though the students are encouraged to use the topics which have been explained in the classes, they widely use the Internet again as a rich source of additional information.

We managed to incorporate two funny chemistry oriented dramas into a lesson. The plays come from the Internet and where created by the native authors without the language learning objective. However, our students find them very entertaining and participate enthusiastically in dramatic readings.

This activity also fits a multilevel class as the students are given the freedom in adapting the sentences. So the less advanced can make their entries less complicated and simpler, while the more advanced ones enjoy the full versions of their roles. Dramatization engages students emotionally and socially, as well as intellectually. The students thoroughly enjoy it, and it leaves strong, memorable impressions which can later help recall and improve learning. Thus at the end of the activity they are able to repeat various catch phrases by heart (e.g. thanks to the plays "Becoming an alcohol – a sad story of good oxygen becoming bad", or "Electrophilic Addition" they will long remember structures as: "My mom has always warned me against organic acids", "It is against the rule. What rule? Markovnikov's rule!" Aren't my electrons good enough for you? "Along comes electron hogging

chlorine" "Keep your precious electrons for yourself, you … !" etc).

The students are encouraged to role play the authentic professional dialogues videotaped for instructional purposes as well as those which are found on the ESL web pages.

After all, we can say that English for chemists is pHun!

P09 VOLATILE DEGRADATION PRODUCTS OF POLYURETHANE FOAMS

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Introduction

Polyurethanes are the world's sixth most abundant synthetic polymer. The most of their production represent flexible polyurethane foams. At the end of their life-cycle they are often deposited on waste dumps, where they degrade under the influence of various environmental factors; photodegradation and hydrolysis are the main routes. After that, their degradation products can be distributed in the environment. In common, synthetic polymers are not prone to environmental degradation. Therefore, they could stay there for a long time. Their degradability can be improved by addition of the biodegradable filler.

The generation of volatile products has been reported from the photo- and thermal degradation of many polymers.[1–4] Complex mixtures of degradation products of this type were for example identified in starch-based polymers[5]. Till now, there is no information about volatile compounds generated during photodegradation of synthetic polyurethane with biodegradable filler. Several studies have been developed on the UV degradation of aromatic polyurethane. Such photo-degradation has already exhibited formation of free radicals, recombination, scission of bonds, crosslinking and oxidation reactions[6,7].

This paper is focused on the identification of volatile photodegradation product of polyurethanes modified by biodegradable filler using Solid Phase Microextraction (SPME) and Gas Chromatography linked to Mass Spectrometry. The SPME method was selected in the experiment for its fastness, simplicity and environmental friendness.

Experimental

M a t e r i a l a n d S a m p l i n g

Polyurethane foam modified with biodegradable filler like carboxymethyl cellulose, acetylated potato starch, cellulose acetate, 2-hydroxyethyl cellulose and wheat protein were prepared at the Institute of Material Chemistry at Faculty of Chemistry, Brno University of Technology.

For sampling of volatile compounds the system consisting of quartz tube with Teflon cover and two SPME holders, the first with polyacrylic fibre (PA) 85 μm and the second with polydimethylsiloxane fibre (PDMS) 100 μm (both Supelco, USA) and UV lamp (high-pressure mercury discharge tube, $\lambda = 254$ nm), was set-up. The system is shown on Fig. 1.

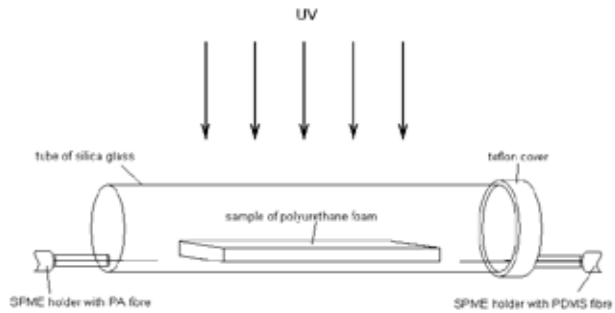

Fig. 1. **System for sampling of VOCs formed by photodegradation of polymer**

G C - M S a n a l y s i s

Separation and identification of volatile compouds from UV-induced polyurethane foam photodegradation was realized using Agilent 6890N gas chromatograph coupled with Agilent 5973 mass selective detector (Agilent Technologies, Germany). HP-5MS column 30.0 m × 0.25 mm × 0.25 μm was used for the separations. The injector and transfer line temperature was 270 °C. The GC oven temperature program for PDMS fibre was: 4 min at 40 °C, then increased at 15 °C min^{-1} to 100 °C, then 8 °C min^{-1} to 270 °C, then 15 °C min^{-1} to 280 °C. For the compounds desorbed from PA fibre the column temperature program was slightly modified: 4 min at 40 °C, increased at 10 °C min^{-1} to 230 °C, then 15 °C min^{-1} to 280 °C. Injection/desorption of analytes from SPME fibres was realized in splitless mode. Helium was used as the carrier gas at a constant flow of 1 ml min^{-1}. Ion source temperature was 230 °C, electron ionization at 70 eV was used. Quadrupole analyzer of the MSD was operated in scan mode within a range 30–550 amu, solvent delay was 4 min. Identification of separated compounds was based on NIST 05 spectral library search.

Results

Seven types of polyurethane foams with different fillers and reference foam without filler were irradiated by UV lamp during this study. Volatile compounds were sorbed on PA and PDMS fibers and desorbed, separated and detected by GC-MS technique.

All identified compound are listed under chromatograms (Figs. 2. and 3.). Many various compounds have been identified.

Group of branched and non-branched aliphatic hydrocarbons containded: pentadecane, hexadecane, heptadecane, 2,6,10,14-tetramethylpentadecane, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (Squalene). These compounds are probably formed by homolysis of bonds in soft part of polyurethane.

Group of ketone and fatty acid esters contained: 6,10-dimethyl-5,9-undecadiene-2-one, methylester of dodecanoic acid and isopropylester of tetradecanoic acid.

By photo-oxidation of alkenes hydroperoxides were formed – 1,3-dioxane and 2-methyl 1,3-dioxane.

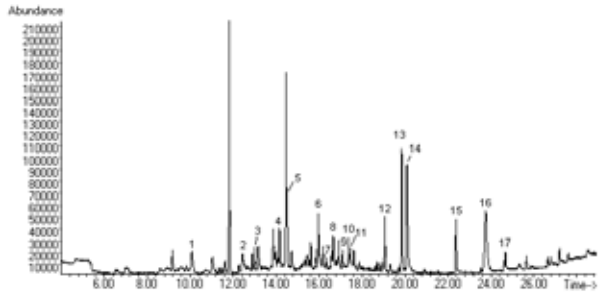

Fig. 2. **Volatile degradaction products of polyurethane foam sorbed on PDMS fibre**
1: 2-methyl-1,3-dioxane, 2: 2,4-diisocyanatotoluene, 3: 6,10-dimethyl-5,9-undecadiene-2-one, 4: 2,5-diterc-butyl-1,4-benzochinone, 5: pentadecane, 6: hexadecane, 7: 2,6-bis(1,1-dimethyethyl)-4-(1-oxopropyl)phenol, 8: 1-methylester dodecanoic acid, 9: 4-decyl-morphline, 10: heptadecane, 11: 2,6,10,14-tetramethylpentadecane, 12: isopropylester tetradecanoic acid, 13: 4-undecyl-morpholine, 14: N,N-dimethyl-1-hexadecanamine, 15: 4-tetradecyl-morpholine, 16: squalene, 17: 4-hexadecyl-morpholine

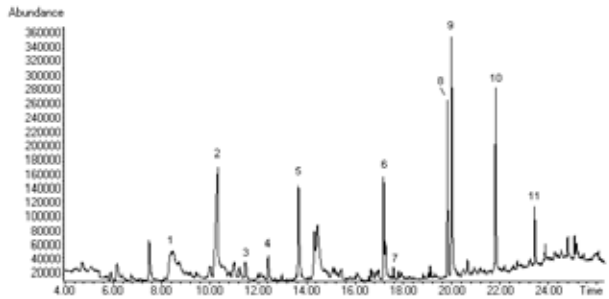

Fig. 3. **Volatile degradaction products of polyurethane foam sorbed on PA fibre**
1: 2-(2-ethoxyethoxy)-ethanol, 2: 2-ethyl-hexanoic acid, 3: 1,3-dioxane, 4: 2-methyl-1,3-dioxolane, 5: 2-methyl-1,3-diisocyanatobenzene, 6: 2,6-bis(1,1-dimethyethyl)-4-(1-oxopropyl)phenol, 7: 4-decyl-morpholine, 8:4-undecyl-morpholine, 9: N,N-dimethyl-1-hexadecanamine, 10: 4-tetradecyl-morpholine, 11: 4-hexadecyl-morpholine

Also nitrogen derivates were detected, namely line of alkyl-substitued morpholines and N,N-dimethyl-1-hexadecanamine.

By the photolysis of hard segment of polyurethane foam 2,5-diterc-butyl-1,4-benzochinone, 2,6-bis(1,1-dimethyethyl)-4-(1-oxopropyl)phenol and 2,4-diisocyanatotoluene (2,4-TDI) were formed.

2,4-TDI is very volatile and toxic. The compound is used as main reagent at synthesis of polyurethane. 2,4-TDI was detected in case of all polymers modified by celulose derivate fillers. It is difficult to say whether that compound is photodegradation product of polyurethane foam or unreacted raw material residue. It will be studied in the next research.

All the compounds were detected in most polyurethane samples. There was observed qualitative change only.

Quantitative differences were found in the case of minority distribution compounds. Their identification using library search in NIST 05 was unsuccessful in most cases. Probably small concentrations of analyte and resulting low-intensity mass spectra could be the reason, or – due to specific character of volatile compound made by irradiation of polyurethane foam – their mass spectra are not included in this library.

PA fibre shows higher selectivity in comparison with PDMS fibre which in opposite gives more complex information about volatile compounds formed during photodegradation of polyurethane.

Conclusions

In this study the SPME method was applied for the identification of volatile compounds formed by irradiation of polyurethane foams modified by biodegradable fillers. A wide range of compounds was detected and most of them were succesfully identified by library search. The identification of the remaining compounds will be a subject of the next research.

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P10 DYNAMIC SIMULATION OF BIOLOGICAL NITROGEN REMOVAL PROCESSES

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Introduction

With rising requirements for protection water resources and thereby for quality of effluent wastewater too, more complex technologies for wastewater treatment are applied as well. These technologies are characterised by more processes with different reaction rates and requirements in term of reaction conditions, interactions and generally by more complex structure of treatment lines. The complexity of these systems is also related to mutual interaction between wastewater treatment plant (WWTP) and others parts of the urban wastewater system, e.g. sewer system and receiving water (Derco and Schütze, 2004). Consequently, designing of processes and technologies and their efficient operation are also significantly complex.

Mathematical models and simulations programs belong to prospective and currently more often utilised tools. They offer a simple way how to analyse changes in technologies at WWTP by optimisation or intensification already existed plants or it can help by designing new WWTPs as well.

The results of application of the Activated Sludge Model No. 1 (ASM1) (Henze et al., 1987) using dynamic simulations for verification of steady state design with regard to dynamic behaviour of the WWTP and for prediction dynamic effluent concentration values are presented.

Dynamic Simulation of WWTP

ASM1 was used for modelling of the biological stage at real WWTP in Nové Zámky (Fig. 1.). Two series of measurements included diurnal variations of wastewater flow and composition in input and output at the biological stage were carried out. The first set of measurement was perfomed in May 2002, when the nitrification process was operated. Because of expected more stringent requirements in effluents, this activated sludge plant was upgraded in 2003 applying a pre-denitrification system. The second measurements were realised after the reconstruction (in March 2007). The results of measurements were transformed into organic and nitrogen pollution fractions according to structure of the ASM1.

For comparison of the experimental and the calculated resultant concentration values in the WWTP effluent were chosen criteria according the Regulation of the Slovak government (2005). The model ASM1 and the default parameter values included in the SIMBA program have been used for performing dynamic simulations. The calculated concentration values for individual pollutants in daily composite samples were calculated based on pollutant mass balancess.

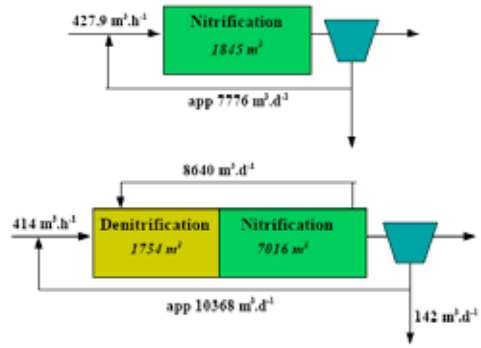

Fig. 1. **Schemes of WWTP setups; Above: WWTP before reconstruction (2002); Below: WWTP after reconstruction (2007)**

Results

Model Suitability

First step of simulations was to verify suitability of ASM1 to describe WWTP. From the results (Fig. 2) it can be concluded that the dynamic simulations describe the experimental effluent values quite well.

Verification of Steady State Disign by Dynamic Simulation

The purpose was to verify the results of conventional steady-state design of the WWTP biological stage (Derco *et al*, 2004) for future upgrading with regard to expected load in 2036. The data for this scenario were obtained from the operator of the WWTP – Západoslovenská vodárenská spoločnosť, a.s. Resulting values (7 mg dm^{-3} ammonium nitrogen and 9.3 mg dm^{-3} total nitrogen) of dynamic simulations are lower than today's effluent standards (10 mg dm^{-3} ammonium nitrogen and 15 mg dm^{-3} total nitrogen). It can be summarised that the steady state design for the WWTP upgrading

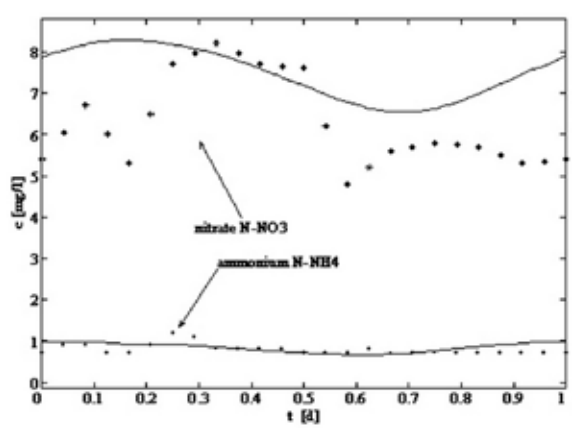

Fig. 2. **Effluent concentrations of ammonium nitrogen at WWTP after reconstruction (*experimental and ⁻calculated values)**

with perspective for 2036 follows also the dynamic effluent standard values. It can be expected that upgraded WWTP will meet current legislation also with regard to dynamics of the processes.

The first task was to investigate what could happen if the reconstruction in 2003 had not been carried out. The effluent concentration values are shown in Table I. The load in 2007 and consequently the effluent concentration values are lower than in 2002 due to industrial activities reduction. This result could lead to the conclusion that there had been no need for the reconstruction of the WWTP in 2003. However, because of the more stringent requirements (Regulation of the Slovak government, 2005) which permits 10.0 mg dm^{-3} for ammonium nitrogen for effluent of WWTP with corresponding load, it was necessary to carry out the reconstruction. Therefore, it should be stressed that the WWTP without this reconstruction would not be able to meet current effluent standards.

The second task was to study what would happen if the pollution load of actual biological stage of the WWTP increased to values expected in 2036. The results of dynamic simulations show that even if the load will increase, the WWTP will be able to meet current effluent requirements.

Table I
Results of simulations

	WWTP 2002	1st task	WWTP 2007	2nd task
plant layout	2002	2007		
load situation	2002	2007	2007	2036
$N\text{-}NH_4$ [mg dm^{-3}]	25.06	20.89	0.81	4.05
$N\text{-}NO_3$ [mg dm^{-3}]	0	0	7.47	3.91

Conclusions

Two time-variable influent and effluent data sets of wastewater flow and composition were measured for different technological arrangements of the WWTP and were applied for dynamic simulations.

It can be concluded from the work that the mathematical model ASM1 is suitable for the description of the process dynamics of the WWTP biological stage. Good fit between experimental and calculated effluent concentration values resulted from performed dynamic simulations.

The results of dynamic simulation confirmed the necessity of the WWTP reconstruction carried out in 2003 in order to comply with actual legislation requirements.

Compliance with actual effluent limits follows for previous steady state design and upgrading of the WWTP when investigating dynamics of the process.

According to results of dynamic simulations it can be expected that the existing WWTP will comply with actual discharge standards also for pollution load as expected in 2036.

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P11 APPLICATION OF A 6-MERCAPTOPURINE FUNCTIONALIZED SORBENT FOR DIFFUSIVE GRADIENTS IN THIN FILMS TECHNIQUE

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Introduction

The diffusive gradients in thin films technique (DGT)[1] is used more than 10 years for determination of kinetically labile metal species in natural waters, soils and sediments[2]. However this technique is at the present time validated for measurement of more than 50 metals, only few attention was applied to measurement of mercury.

As we reported in our previous studies[3,4], combination of agarose diffusive gel together with the sorption gel containing resin with thiol functional groups is the best choice for measuring of mercury by DGT. Nevertheless, at the present time there is almost no resin of this type for direct use in DGT on the market. For DGT, strictly defined size of resin about 100 μm is used. As the resins available on the market have particle size about 1 mm, there is need to crush their particles and separate the required fraction by sieving. This activity can seriously contaminate the resin or it can change its properties.

In presented study we try to prepare needful resin by our self in laboratory. The Iontosorb AV resin (Iontosorb, Czech Republic) with particle size 50–100 μm was used in advance. This resin contains free amino group which can be easily diazotated (Fig. 1.). Resulted diazonium salt can be subsequently used for raction with specific reagent containing thiol groups. In this work we used 6-mercaptopurine as this reagent. New modified Iontosorb AV was characterised in laboratory and then it was used to prepare sorption gels for DGT technique. The performance of DGT with modified Iontosorb AV was then tested in model mercury solution.

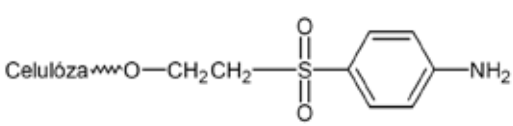

Fig. 1. **Chemical formula of Iontosorb AV**

Experimental

Modification of Iontosorb AV Resin

Iontosorb AV was modified using diazotation and copulation reactions[5,6]. The amount of 5 g Iontosorb AV was washed with hydrochloric acid and then with ultrapure water to neutral pH. After washing, diazotation was performed at 0–5 °C using 1M solutions of hydrochloric acid and sodium nitrite. Diazotation was stopped after adding of 40 ml of reagents. Yellow product was filtered out and washed several time with ultrapure water. Diazonium salt was then placed to the continuously mixed and cooled reactor containing 3.5 g of 6-mercaptopurine dissolved in 10% (v/w) sodium carbonate. After 24 h new red-brown product was filtered out, washed several time with ultrapure water and dryed in exicator.

Analytical Control of New Functionalised Resin

Qualitative test was done using an infrared spectroscopy (Impact 400, Nicolet, USA). Infrared spectrum of new functionalized resin was compared with blank infrared spectrum of Iontosorb AV.

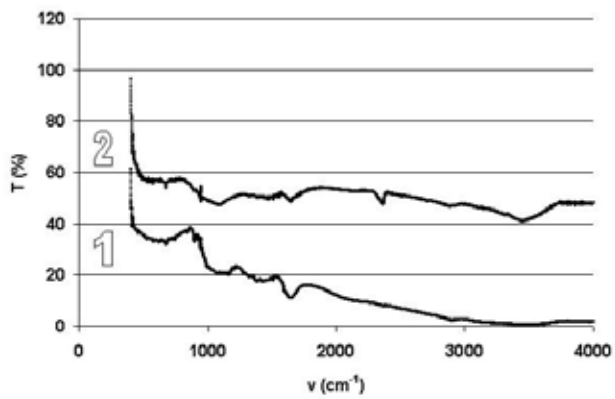

Fig. 2. **Infrared spectrum of Iontosorb AV (1) and new functionalized resin (2)**

Quantitative test of thiol groups was done by iodometric titration at pH ~ 9. To 50 mg of modified Iontosorb AV, 25 ml of 0.001M potassium iodine was added, and after 5 min of shaking and 30 min of standing in the dark, resulted solution was titrated by 0.001M ammonium thiosulphate.

Testing of New Functionalised Resin for Use in DGT Technique

Sorption gels for DGT technique were prepared by incorporation of modified Iontosorb AV into the agarose gel. The amount of 0.2 g of modified Iontosorb AV was added to a hot agarose solution (4% (v/w)) and the hot solution was casted between two glass plates separated by 0.4 mm plastic spacers. The disks 2 cm in diameter were cuted out from the gels after cooling down and washig of the gel sheets.

Diffusive gels were prepared by similar way, the only one difference was that clear hot agarose solution (3% (v/w)) and 0.8 mm plastic spacers were used.

The agarose sorption and diffusive gels were used to assembly the DGT unit. Four DGT units were exposed in a 5 dm^3 beaker containing well mixed 10 $\mu g\,dm^{-3}$ mercury solution for 3 h to perform basic DGT test. Simultaneously to the exposition of DGT units, mercury concentration in the solution was measured by atomic absorption spectrometry (AMA 254, Altec, CZ).

Results

Sucessful incorporation of 6-mercaptopurine to Iontosorb AV was proved by chage in resin colour from white to red-brown. Infrared spectroscopy has shown a new peak in Iontosorb AV spectra with wave nuber 2,362 cm^{-1} (Fig. 2.). This is a characteristic peak for thiol groups. The amount of thiol groups was determined by iodometric titration to be 0.5 mmol g^{-1}. This should be enough to prevent the saturation of functional groups when DGT sampling units are deployed in natural waters. New resin was successfully incorporated into the agarose gel. The agarose gel as a support for resin was chosed because of problems associated with commonly used polyacrylamide gel. In polyacrylamide gel, the new resin aggregated and it was difficult to prepare homogenous gel. In agarose gel there was no problem with aggregation of resin particles. After optimalization of sorption gel preparation procedure, basic DGT test was performed. Results from this experiment are shown in Table I. It can be seen that the difference between real mercury concentration measured by AAS and mercury concentration calculated from accumulated mass of mercury in resin gels extracted from DGT sampling units is less than 10 %. This result matches the requirements of DGT Research Ltd. for right function of DGT.

Table I
Results from basic DGT performance test

DGT concentration [μg dm^{-3}]	Real concentration [μg dm^{-3}]	Difference [%]
7.5	8.0 ± 0.4	6
8.2	8.0 ± 0.4	3
8.7	8.0 ± 0.4	8
8.2	8.0 ± 0.4	3

Conclusions

This study proved that the 6-mercaptopurine functionalised Iontosorb AV is useable for measuring of mercury in natural waters by DGT and it provided useful informations for further experiments in which better characterization of new sorbent will be carried out and more extensive investigation of its use in DGT will be done. For example, experiments in solutions containing natural ligands and experiments in real sytems will be realized during the next years and a comparison with other sorbents usable in DGT will be done.

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P12 THE DETERMINATION OF METHYLMERCURY IN WATER ECOSYSTEMS

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Introduction

Alkyl mercury compounds belong to a group of organometallic compounds with a high bioaccumulation potential. They are formed from inorganic forms in a methylation process and exhibit 100 times higher toxicity than inorganic forms[1]. Methyl mercury is a neurotoxin which attacks the central nervous system (CNS). The most frequent pathway by which methyl mercury enters the body is through the gastrointestinal tract which may absorb up to 95 % of methyl mercury received by a man via fish meat. It may also penetrate through the skin. It is well soluble in fat which explains its transport through blood-brain barrier and diffusion into cell membranes. It also passes the foetal placenta; the risk of damage to the foetus occurs at a mercury concentration in hair as low as 15–20 mg kg^{-1}. The tolerated dose of mercury in man is 33 μg per 70 kg of body weight[2,3,4]. Methyl mercury was responsible for a large number of intoxications in the past. For example, fish living in water polluted with waste from a chemical company producing chlorine caused intoxication by methyl mercury in Japan while mercury intoxications in Iraq were caused by the grain treated with methyl-mercury-containing fungicides that was originally intended as seed[5]. Methyl mercury is the most common organic form occurring in biological systems. It is soluble in water and is relatively stable. It passes biological membranes easily and has a long half-time of decomposition up to 70 days[4]. Methyl mercury has the highest partition coefficient K_{ow}, which explains its high affinity towards fat. Methylation is one of the mercury's most important environmental reactions. Methylation takes place in the sediment as well as in sea water and fresh water. The fastest rate of methylation was observed on the sediment's surface that was in contact with water [6,7].

Experimental

The optimization of the method was carried out using the Certified Reference Material CRM 464; tuna fish containing a total amount of THg = 5.24 μg g^{-1} and methyl mercury MeHg = 5.5 μg g^{-1}. The sample weighed 0.1 g. A total of 5 samples were extracted and each sample was subjected to three parallel measurements. Extraction was performed according to the published method[8]. Gas chromatography was used as the final analytical method. The results of parallel measurements were evaluated as mean values and their standard deviation was calculated.

Table I
The conditions of the GC/μECD analysis

Parametrers of GC/μECD	
Column	DB-608, 30 m × 0.530 mm × 0.5 μm
Injection	splitless
Injector temperature	250 °C
Carrier gas	He,3 ml min^{-1}, constant flow
Detector temperature	250 °C
Make-up gas	N2, 20 ml min^{-1}

Results

The Certified Reference Material was used particularly for the determination of metrological parameters of the method. The mean recovery of extraction was found to be 64.9 ± 2.0 %. The lower recovery rate as compared to the literature(ref.[8]) could be caused by the certified reference material used which just passed the expiry date. As a result, the content of methyl mercury might differ from that provided in the certificate. Fig. 1. shows the chromatogram of the analysis of MeHg isolated from the certified reference material.

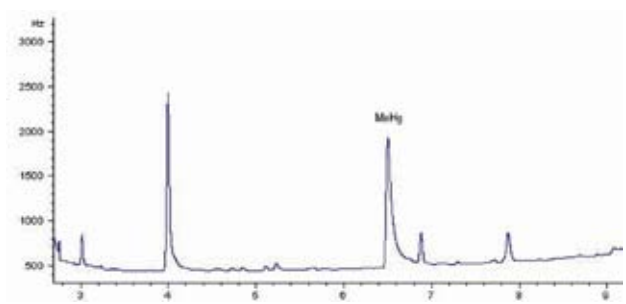

Fig. 1. **Chromatogram for the certified reference material**

Table II contains the results of recovery from individual parallel measurements of methyl mercury levels in the CRM (Marked as 1–5).

To determine the accuracy of methyl mercury determination using GC/μECD, 5 parallel measurements of the certified reference material were carried out. The results are provided in Table III.

Table II
Recoveries for 5 parallel samples of certified reference materials. Declared value of MeHg – 5,500 [ng g^{-1}]

CRM	Mean peak area	c [ng ml^{-1}]	MeHg [ng g^{-1}] exper.	Recovery [%]
1	6,163.81	40.05	3,559.60	64.72 ± 0.51
2	7,030.21	45.67	3,653.96	66.44 ± 3.80
3	6,938.18	45.08	3,606.12	65.57 ± 1.71
4	6,884.60	44.73	3,578.27	65.06 ± 0.61
5	6,625.99	43.05	3,443.86	62.62 ± 3.59

Table III
The accuracy of methyl mercury determination using GC/μECD

	Mean level [ng g^{-1}]	RSD [%]
MeHg	3,568.36	1.79

Conclusions

The method of determining the level of methyl mercury in fish muscles using GC/μECD was modified. The optimization of the method was performed using the Certified Reference Material. On the basis of recovery and accuracy, it was concluded that the test procedure without sample pre-treatment can be applied with this method of analysis.

This work has been supported by by the grant No. 6215712402 given by MSMT.

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P13 ECOTOXICOLOGICAL TESTING AND TEST METHODS OF CHEMICALS

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Introduction

Ecotoxicity involves the identification of chemical hazards to the environment. The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) describes testing for hazards to the aquatic environment in Part 3, Chapter 3.10 (UNECE, 2004a). Annex 8, Guidance on Hazards to the Aquatic Environment (UNECE, 2004). Ecotoxicity tests can be classified into two following categories: standard and alternative tests.

The ecological testing according to US EPA and UNECE includes many tests; however, several methods of ecotoxicological testing are used in the Czech Republic. For the typical standard and alternative aquatic ecotoxicological tests crustacea (*Daphnia magna, Thamnocephalus platyurus, and Artemia salina)*, aquatic plant (*Lemna minor*), algae (*Desmodesmus subspicatus, Scenedesmus subcapitatus, Selenastrum capricornutum)* are used. In accordance with OECD, UNECE and Czech legislation the standard tests include these organisms in following tests – *Daphnia magna* (OECD Test No. 202: Daphnia sp. Acute Immobilizations Test, EN ISO 6341:1996), *Desmodesmus subspicatus* (OECD – Test No. 201: Alga, Growth Inhibition Test, ISO 8692:2004). The aquatic organism *Lemna minor* (OECD Test No. 221: Lemna sp. Growth Inhibition Test, ISO 20079:2005) is application in OECD and ISO standard methods for the testing of chemicals.

Experimental

The general principle of ecotoxicological tests is the determination of effective concentration (EC50), eventually lethal concentration (LC50) or inhibition concentration (IC50). The limit test corresponds to one dose level of 100 g dm^{-3}. A stepwise procedure involves four steps: the limit test, confirmatory test, basic test and definitive test.

These types of studies produce end points such as LC50, EC50 and NOEC. EC50, LC50 and IC50 are the effective concentrations (i.e., the concentration of material in water that is estimated to produce a specifically quantified effect to 50 % of the test organisms). The EC50 and its 95 % confidence interval are usually derived by statistical analysis of a quantal, "all or nothing", response (such as death, fertilisation, germination, or development) in several test concentrations, after a fixed period of exposure. End point means the variables (i.e., time, reaction of the organisms) that indicate the termination of a test, and also means the measurement(s) or value(s) derived, that characterise the results of the test (EC50). LOEC (lowest observed effect concentration) is the lowest concentration tested causing a statistically measurable effect to the test system. NOEC (no observed effect concentration) is the highest concentration tested causing no statistically measurable effect to the test system. The parameters LOEC and NOEC may be statistically determined.

The aim of statistical analysis of ecotoxicological data is to obtain a quantitative concentration-response relationship by regression analysis. For this purpose many models could be used such as: linear interpolation, polynomial regression, log–logistic regression, probit model, Weibull model, Dunnett test, William test, Jokheere-Terpstra test.

Ecotoxicological Tests for Determination of Ecotoxicity of Standard K_2CrO_4

The standard chemical K_2CrO_4 was used for the determination of ecotoxicity, for th estimation of sensitivity of testing organisms *Dapnia magna Strauss*, (Daphtoxkit F™ magna – standard test) *Thamnocephalus platyurus* (Thamnotoxkit F™ – alternative test) and *Lemna minor* (standard test OECD) and for validation and comparison of obtained results with the declared values. Table I performs the basic parameters of tests in coordinance with the directive.

Table I
The parameters of tests

Parameters	Daphtoxkit F™	Thamnotoxkit F™	*Lemna minor*
c [mg dm^{-3}]	0.32–3.20	0.032–0.320	10–160
T [°C]	22 ± 2	20 ± 2	24 ± 2
I [lx]	6,000	4,000	–

Results

On the base of analysis of obtained ecotoxicological results for studied tests the values of EC50, LC50 and IC50 were determined for standard chemical K_2CrO_4. The experimental data with the basic statistic characteristics are summarized in Table II. The EU-Directive 93/67/EEC classifies substances according to their ecotoxicological value (EC50). On the base of obtained data from ecotoxicological tests (crustacea tests Daphtoxkit F™ and Thamnotoxkit F™) the study chemical belongs to very toxic for aquatic organisms. The studied chemical K_2CrO_4 is also toxic to aquatic organisms for macrophyta *Lemna minor*.

The *Thamnocephalus platyurus* was found as the test organism of highest sensitivity with LC50 = 0.092 ± 0.002 mg dm^{-3}. The effective concentration (EC50/24 h) from Daphtoxkit F™ for exposure 24 hours was 0.99 ± 0.022 mg dm^{-3} and the effective concentration (EC50/48 h) was confirmed 0.79 ± 0.031 mg dm^{-3} The inhibition concentration for *Lemna minor* (IC50/168 h) was calculated from average specific growth rate and confirmed via percent reduction in yield, IC50/168 h was equal to 20.52 ± 0.399 mg dm^{-3}. For validation and confirmation of experimental data the probit model was used for determina-

tion of ecotoxicological parameters. Calculated values from probit model and experimental values were in very good correlation with declared value of tests.

Table II
The ecotoxicity of K_2CrO_4 (n = 9; concentration in mg dm^{-3})

	Daphtoxkit F™ EC50/ 24 h	EC50/ 48 h	Thamnotoxkit F™ LC50/24 h	*Lemma minor* IC50/168 h
Declared values	1.03	0.75	0.10	–
Experim. value	0.99	0.79	0.092	20.52
Probit model	0.96	0.69	0.093	–
Median	0.99	0.75	0.093	20.00
s	0.0286	0.0411	0.0035	0.5200

Conclusions

The ecotoxicity of the tested chemical was confirmed by the application of ecotoxicological tests. In the majority of cases, on the base of the obtained results of the study tests with chemical, the tests perform very suitable way for the study of ecotoxicity and can be used for evaluation of ecotoxicity of other chemicals and wastes. This study confirmed declared value and all data were statistical evaluated in detail, and the sensitivity of testing organism was also confirmed.

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P14 INDOOR AEROSOL EXAMINING

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Introduction

Aerosol particles are generally considered to be one of the principal indoor risk factors. Operation, number and behavior of occupancy i.e. type, emission intensity and amount of indoor contamination sources (building materials, combustion processes, smoking, cleaning)[1,2] determine temporal and spatial variations of indoor aerosol distribution. The particulate matter (PM) cause the negative health effect, when they are inhaled and deposited in the respiratory tract[3].

This paper is primarily concerned with suspended PM_{10} concentrations and indoor settled PM monitoring with regard to the chemical composition and shape of PM_{10} particles.

Experimental

M e t h o d s

Suspended particulate matter investigation was focused on thoracic fraction PM_{10} monitoring in various types of residential and non-residential buildings. Measurement includes integral particles sampling onto a collection material (membrane filter Synpor 0,8 μm pore size, 35 mm in diameter) by sampling equipment VPS 2000 (Envitech, Trenčín) at air flow of 960 $dm^3 h^{-1}$ during sampling period of approximately 24 hours. The sampling was carried out in the middle of the room at the height of 1,500 mm from the floor. The windows and the door were closed during the monitoring period. The particulate mass concentrations were determined by gravimetric method from the increase of filter weight. Because of minimisation of humidity interference, the filters were dried at 105 °C for 8 h before and after sampling and than were equilibrated at a constant temperature and humidity (e.g. 20 °C and 50 % RH) for 24 h before and after sampling.

The monitoring of indoor settled PM was performed in selected flat building. Sampling was carried out by passive methods based on PM settling into Petri dishes during 28 days. The particle surface concentrations were determined by gravimetric method.

The PM_{10} samples were characterized by scanning electron microscopy/energy-dispersive X-ray analysis (SEM/EDX) and atomic absorption spectroscopy (AAS) as the main techniques. Particle morphology was determined by SEM on the equipment Tesla BS 340. The elemental EDX analysis were carried out on the micro-analytical system LINK ISIS 300 (Oxford Instruments) operating in secondary mode at a potential 25 kV and at extension 600–30,000. The chemical analysis of the selected metals samples content was realised by SpectrAA-30 (Varian).

Results

The results of indoor PM_{10} monitoring in different types of residential and non-residential buildings are illustrated as average mass concentrations in the Fig. 1.

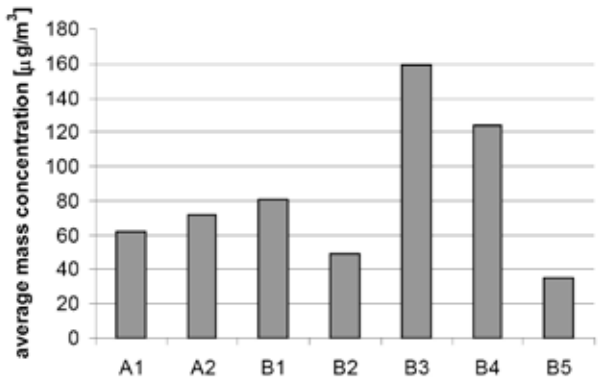

Fig. 1. **The average mass concentrations of PM10 in residential (A) and non-residential (B) buildings: A1 – single family residences, A2 – flat-residences, B1 – school buildings; B2 – offices; B3 – commercial buildings; B4 – buildings for culture and ntertainment, B5 – hospitals and sanitary facilities**

The highest mass PM_{10} concentration was observed in non-residential public buildings. The PM_{10} hygienic limit for indoor air in Slovak republic – 50 $\mu g\, m^{-3}$ was exceeded in all monitored types of residential and non-residential buildings excepting B2 (offices) and B5 (hospitals). The concentrations of settled PM were measured at various heights and were ranged from 21.0 $\mu g\, cm^{-2}$ to 86.6 $\mu g\, cm^{-2}$. The trend of gradual decreasing of particulate matter occurrence with the raise of height was observed (Fig. 2.).

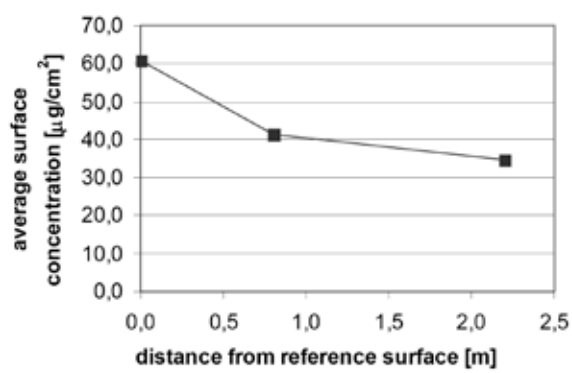

Fig. 2. **Average mass surface concentration of settled PM in dependence on the height**

The mass concentrations of metals in PM_{10} samples investigated by AAS are summarised in Table I.

The obtained mass concentrations of metals in indoor particle samples correspond with those of in typical urban aerosol[5]. The individual particles in irregular shape of the various particle size as well as particles aggregates were observed on the SEM micrographs (Fig. 3.).

Table I
The mass concentrations of metals

Element	Average [μg m^{-3}]	Minimum [μg m^{-3}]	Maximum [μg m^{-3}]
Cu	0.704	0.03	3.38
Pb	0.094	0.007	0.666
Cd	0.094	0.002	0.625
Ni	0.050	0.007	0.29
Cr	0.058	0.007	0.35
Zn	0.408	0.08	2.01
As	0.007	0.0001	0.019
Fe	1.818	0.36	9.23
Sb	0.115	0.0001	1.47

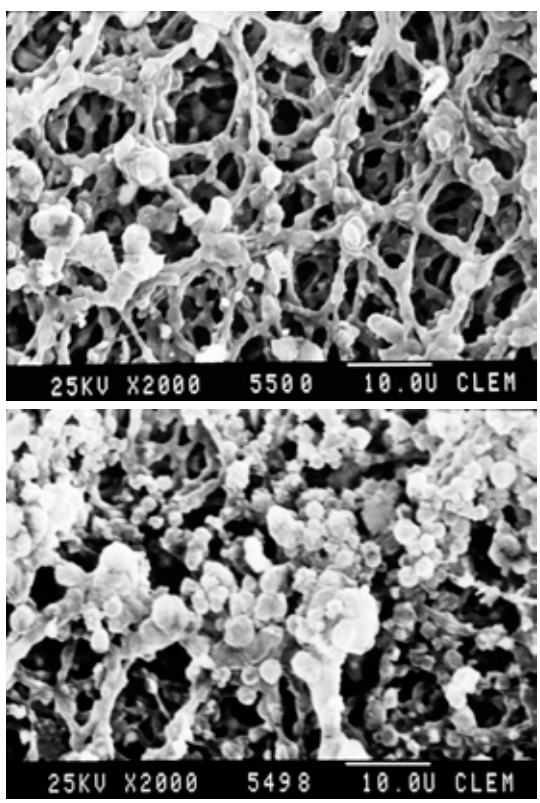

Fig. 3. **SEM micrograph of particles**

The majority of PM_{10} particles are non-spherical in shape with strong division of the surface. The occurrence of the spherical particles as well as of fibrous particles was not obvious[4].

The energy-dispersive X-ray system interfaced to the SEM provides preliminary information on the elemental composition of the samples. Fig. 4. represents EDX spectrum of the selected solid aerosol sample. The principal inorganic elements constituting the particles in order of decreasing peak intensity were Ca ≈ Si > O > Al > C > Mg > Fe > Cl > Na ≈ K.

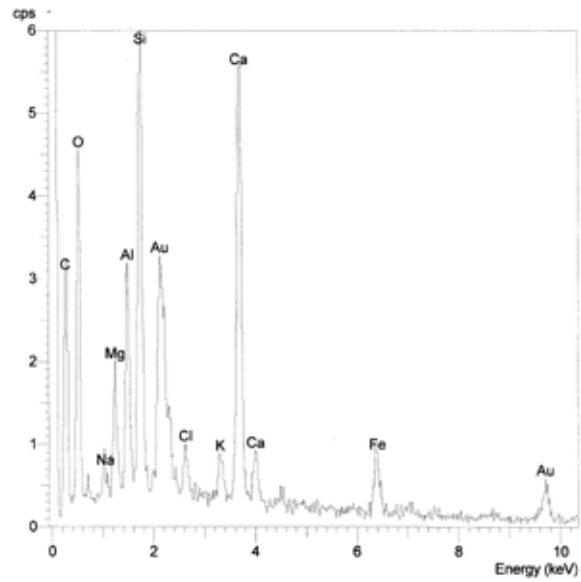

Fig. 4. **EDX spectrum of suspended PM_{10}**

Conclusions

High indoor PM_{10} concentrations exceeding the hygienic limit (excepting offices and hospitals and sanitary facilities) were found out in this study. The trend of gradual decreasing of settled particulate matter concentration with height rising was observed. The determined metal mass concentrations in PM_{10} correspond with the typical metal concentration range of the urban aerosol. SEM investigation refers the presence of majority particles non-spherical in shape, in primary as well as secondary form (aggregates).

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P15 ATMOSPHERICAL DEPOSITION AND IMMISSION SITUATION IN THE NIŽNÁ SLANÁ AREA

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Introduction

Owing the positive results of geological survey, the Nižná Slaná region has become during last 35 years the most important basis of Fe-ore. Potential of industrial siderite is 63 mil. t[1,2].

The biggest of the polution sources is the chimney which is 120 m high. The dust fallout was monitored by means of 17 sampling points located maximally up to 8 km from main source of pollution. The pollutants have municipal as well as industrial origin[5]. The range of plant emission by chemical data processing and by their comparision with dust deposition was investigated. In the present time the communal and the natural sphere causes the air pollution[3,7].

Experimental

Samples of the dust deposition were taken in monthly intervals from seventeen sampling places in the intervals of 30 ± 3 days. To take the samples of the dust deposition plastic sedimentation containers having a cylinder shape with the internal diameter of 12.5 cm were used and located on two holders in the height of 2.5 to 3 m[1,4,7]. The containers were filled with 250 ml of distilled water with additives of substances preventing creation of algae in the summer period and antifreeze additives in the winter period. After sampling the content of the containers was quantitatively transferred to the evaporating dishes and evaporated. To remove the organic mass the evaporation residue was annealed at the temperature of 450 ºC. The temperature was selected not to cause degradation of present carbonates and so possible distortion of gravimetry of the dust deposition. The gravimetry was made separately for each of the containers[6]. The result for each month and individual sampling places is calculated in units of $g\,m^{-2}(30\ days)^{-1}$ and it is the average of alues from two exposed containers. The inorganic part from twelve months set by annealing was cumulated into one sample and after mineralization analysed for individual monitored elements using the AAS method and the SpectrAA – 30 VARIAN unit[4,7].

Results

Localisation of the sampling places of deposition is shown in Table I.

The results are the values acquired from deposition samples after drying and annealing. The specific values of dust fallout are shown on Table II. As it is evident from these results, the highest values of the dust fallout were recorded in summer months and the vegetation period and that is reflected in the percentage representation of the annealed organic part.

Table I
Localisation and the samples quantity in the year of 2007

Number of locality	Locality	The samples quantity
1	Vlachovo – swimming pool	12
2	Gočovo – playground	11
3	N. Slaná – colony	12
4	Above plant	12
5	Before plant	12
6	N. Slaná – agrarian cooperative	12
7	N. Slaná – playground	12
8	Direction to Kobeliarovo	12
9	Crossroad – Štítnik	11
10	Henckovce – before village	12
11	Henckovce – upper part	12
12	Henckovce – cemetary	12
13	Henckovce – near railway	12
14	Henckovce – lower part	12
15	Between H. a G. Polomou	11
16	Gemerská Poloma	12
17	Betliar	12

According to Table II and Fig. 1. the highest average values were recorded on sampling places no. 7 and 5 in 2007 being directly influenced by the dust deposition from the spot source of the plant and localized in the central part of the valley (4.40 an 2.77 $g\,m^{-2}$ $(30\ days)^{-1}$ accordingly). Just to compare, the average value from the sampling place no. 8 that is localized relatively close to the plant, but on the west side of the valley (west side of Nižná Slaná in the direction to Kobeliarovo) achieves only 0.57 $g\,m^{-2}(30\ days)^{-1}$. Some of the increased values mainly from the summer period need to be ascribed also to the extremely dry weather in 2007 and blowing out of the inorganic particles from the uncovered horizonts of the farmland.

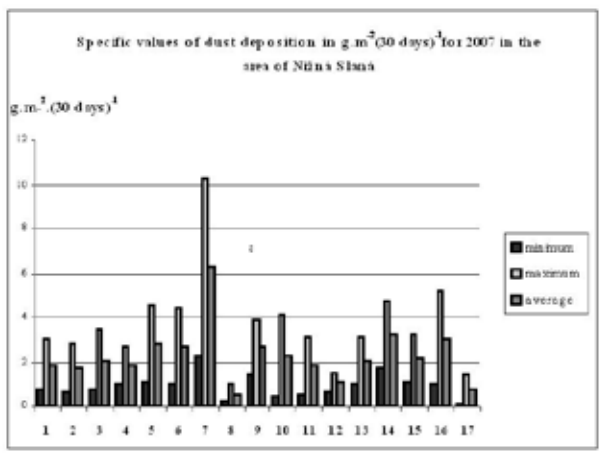

Fig. 1. **Specific values of dust deposition in $g\,m^{-2}(30\ days)^{-1}$**

Table II
Specific values of dust deposition in $g\,m^{-2}\,(30\ days)^{-1}$

No.	Locality	After dying			After annealing		
		min.	max.	aver-age	min.	max.	aver-age
1	Vlachovo – swimming pool	1.08	4.72	2.90	0.73	3.00	1.87
2	Gočovo – playground	1.07	12.13	6.60	0.70	2.82	1.76
3	N. Slaná – colony	1.28	5.76	3.52	0.72	3.43	2.07
4	Above plant	1.32	8.29	4.80	0.98	2.71	1.84
5	Before plant	2.10	5.24	3.67	1.10	4.52	2.81
6	N. Slaná – agranian cooperative	2.65	8.80	5.72	0.93	4.41	2.67
7	N. Slaná – playground	3.01	13.22	8.12	2.29	10.23	6.26
8	Direction to Kobeliarovo	0.34	3.97	2.16	0.18	0.98	0.58
9	Crossroad – Štítnik	1.69	9.82	5.76	1.38	3.92	2.65
10	Henckovce – before village	0.79	9.11	4.95	0.42	4.08	2.25
11	Henckovce – upper part	0.94	8.65	4.79	0.55	3.10	1.83
12	Henckovce – cemetary	0.96	5.08	3.02	0.61	1.49	1.05
13	Henckovce – near railway	1.21	8.62	4.92	0.94	3.12	2.03
14	Henckovce – lower part	2.07	12.71	7.39	1.68	4.79	3.24
15	Between H. and G. Polomou	1.67	6.07	3.87	1.09	3.23	2.16
16	Gemerská Poloma	1.37	7.83	4.60	0.96	5.16	3.06
17	Betliar	0.55	6.37	3.46	0.13	1.42	0.77

Conclusion

Monitoring of the dust deposition in 2007 year has shown a relative increase of dustiness in the monitored area influenced by the activity of the Siderit plant. The increased impact can be observed in the area of the farm where the smoke plume touches the terrain immediately.[4] Specific sources of gaseous and solid substances exhaled to the move with air flows in the heights of 100 – 250 m. The research in this area will enable to highlight the positive activity of the plant management in relation to the public in the interest of improvement of environment quality in the given area.

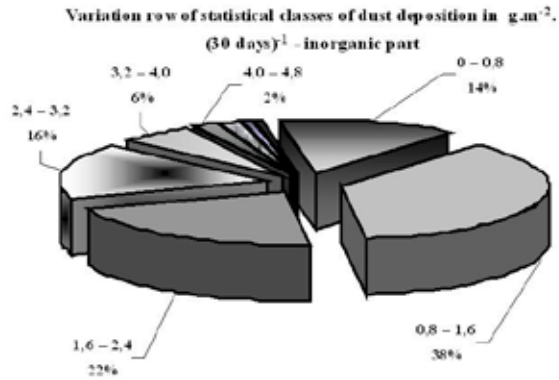

Fig. 2. **Variation row of statistical classes in $g\,m^{-2}(30\ days)^{-1}$ - inorganic part**

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P16 THE NATURAL AND ANTHROPOLOGICAL CONTAMINATION SOURCES OF THE HALČIANSKY WATER RESERVOIR

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Introduction

Many technical sights, as an integral part of a landscape structure, are at the same time its dominant features as they are directly related to its history. In the Banská Štiavnica region they are mostly related to mining traditions. Importance of water reservoirs, also called "tajchy", in the region stems their recreational utilisation. The cleanliness of water is therefore a key issue[1].

This study has focused on the identification of possible natural and anthropogenic sources[2] of pollution in the model territory of the Halčiansky water reservoir and consequently on its contamination. Water reservoirs built to provide the water energy for driving mining machinery are a part of a mining history. As such they are a typical landscape-ecological component of the Banská Štiavnica landscape. Because of their significance they are listed on The State List of Cultural Monuments as sights of technical development and on The World Catalogue of Water Reservoirs.

Experiment and Methods

In the model territory of the Halčiansky Water Reservoir, conditions of potential movement of water and related contaminants were analysed in relation to natural and anthropogenic sources of contaminants with the use of GIS (Geomedia Professional) tools. The aim of the analysis was to determine the vector of the transport and representative sampling points. Based on the system analysis of the above conditions, the selection of sampling points was proposed with the sampling points for the transport system set as transparent. Samples of water taken at the representative points (period 2006–2007) were analysed in situ by spectrophotometer (Hach DR 2000, Horiba) and related to an assumed model of contamination. The results of physical-chemical analysis were further compared with the expected transit and interpretation of the contamination in the territory.

Results and Discussion

The following characteristics have been derived from the results and water quality monitoring:

(i) Water reaction, conductivity, turbidity, temperature, salinity, nitrates (1.3–2.2 mg dm^{-3}) and amount of chlorides (1.6–9.3 mg dm^{-3}) and sulphates (18–33 mg dm^{-3}) complied with the norm No. 490/2002 Z. z. during all seasons, and no measured value exceeded the norm.

(ii) An increase of ammonia, nitrites, phosphates concentrations and of chemical consumption of oxygen exceeding the limit value set by the norm No. 491/2002 Z. z. was recorded during all four seasons.

- Free ammonia – concentration values of NH_3 were in a range of 0.07–1.07 mg dm^{-3} them exceeding the limits of the norm (< 0.3 mg dm^{-3}). In the vicinity of the sampling points 2 and 3 there were grazing grounds foraged by cattle and sheep and an agricultural land fertilized by ammoniac, sulphate ammonia or liquid manure, increasing concentration of ammonium salts in water. Cattle excrements and fertilizers are entering water reservoir by the run-off flowing down the surrounding slopes. The directions of run-off conditions also confirm this. Another source of pollution at the sampling points 5 and 6 was stabled cattle and cesspools from the residential or recreational areas.
- The most distinctive representation of pollution in the form of nitrites 0.004–0.226 mg dm^{-3} was detected in spring when the concentration of those contaminants exceeded the value set up by the norm almost eleven times. Mutual comparison of sampling points indicated the highest concentration of nitrites at the sampling sites 3, 4, 5 and 6. Nitrites are products of biochemical oxidation of ammonia and to lesser extent products of the reduction of nitrates. Their sources are household sewage and wastewater.
- There were also excessive concentrations of phosphates 0.6–2.1 mg dm^{-3}, which exceeded limits set up by the norm five times, with maximum concentrations registered in autumn at the sampling site 2. Main sources of phosphates are household sewage and wastewater together with dead bodies of plants and animals.
- Chemical consumption of oxygen 10–2,800 mg dm^{-3} had the highest value in winter, at the sampling site 1 (the dam), exceeding the limit value set up by the norm eighty times. Organic water pollution can be both of natural (leaches from the organically rich soil, forest, peat bog, etc.) and artificial origin (pesticides, fertilisers).

The above results have shown that the selection of sampling points based on the system analyses and GIS outcomes confirmed assumptions of physical-chemical analysis of water in natural and residential zones. The exceptions were nitrates and chlorides concentrations, in case of which the higher values were expected especially in the agricultural areas or residential zones.

Conclusions

Results of potential conditions of contaminants in the territory of the vector movement of water and gravitation confronted with results of physical-chemical analyses show the relevance of the model. This will enable an effective

and rational selection of sampling places in the future, ensuring the representativeness of sampling in the professional analysis of water contamination.

This work has been supported by grant VEGA 1/3276/06.

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P17 BIOMODIFIED FORMS OF NATURAL ZEOLITE AND THEIR ENVIRONMENTAL APPLICATION

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Introduction

The increasing levels of heavy metals in the environment represent a serious threat to human health, living resources, and ecological systems. Mobile and soluble heavy metal species are not biodegradable and tend to accumulate in living organisms, causing various deseases and disorders. Amongst various treatment methods, ion exchange and sorption seem to be the most attractive in case those nontoxic, low cost zeolites are used.

Natural zeolite of clinoptilolite type (CT) from East Slovakian deposit in Nižný Hrabovec has been studied with respect to its feasibility of application in environmental area in combination with biotechnological methods.

This recent investigation presents a continuation of our previous study[1,2] of the decrease of content of heavy metal and other toxic compounds (polychlorinated biphenyls-PCB) in plants growing on heavily contaminated soils in industrial areas. Natural zeolite as well as zeolitic fertilizers was used in this study. The results of study of growing certain agricultural plants in contaminated soils with varying dosages of natural zeolite (CT), zeolitic fertilizer and standard NPK fertilizer confirmed the favorable influence of both zeolite and the zeolite based fertilizer. Analysis of plant material showed that the lowest content of heavy metals (Zn, Cu, Pb, Cd and Cr) as well as of PCB was found in plants grown in contaminated soils with the application of CT. The content of heavy metals and PCB was lower almost of a half in comparison with plants grown on untreated contaminated soils[2]. Plants grown in contaminated soils with the addition of zeolitic fertilizer showed a somewhat higher content. Natural clinoptilolite by ion exchange of heavy metals and sorption of toxic substances into its cavities and channels blocked their reception into the plants.

The aim of the recent study is the enlarging of clinoptilolite sorption surface by the effect of microorganisms and obtaining more efficient results of its application in the process of reducing the residual content of heavy metals and other toxic compounds in industrial contaminated soils.

According to the literature[3–5], certain species of microorganisms have been found to absorb surprisingly large quantities of heavy metals. The removal of heavy metals from municipal and industrial wastes by biological treatment systems has continued to be of interest. Bacterial surfaces have great affinity to sorb and precipitate metals resulting in metal concentration on the surface. All microbes, which expose negatively charged groups on their cell surface, have the capacity to bind metal ions. Complexolysis is a process corresponding to microbial formation of complexing and chelating agents that solubilize metal ions. The microorganisms are able to transform toxic compounds to less toxic. Biosorption of copper (II) ions by Thiobacillus ferrooxidans were studied and is shown to be an effective bacterial bioaccumulation process[3]. Acidithiobacillus and Thiobacillus cultures are used for biological reduction of chromium (VI)-containing wastes[4]. The attention is paid also to the utilization of combination of microorganisms and microporous materials (active carbon, zeolite) to achieve better sorption properties for adsorption of toxic compounds[5].

Our recent study is aimed to biomodification of the surface of natural zeolite of the clinoptilolite type using the microorganisms Thiobacillus ferrooxidans. Studied are also model forms of zeolites containing copper ions, and the influence of microorganisms on the biosorption of these ions as well as other changes connected with metabolic activity of the microorganisms present. The main motivation of this study is the remediation of soils contaminated with high concentrations of the residual of heavy metals and other toxic compounds.

Experimental

In order to study modified forms of natural zeolite, the natural zeolite of clinoptilolite type (CT) was used from the Eastern Slovakia deposit in Nižný Hrabovec. Two various granulometric classes were taken for the experiments: fine-grained one of the particle size up to 200 μm, denoted as CT1, and coarse-grained of the particle size 0.4–0.6 mm, denoted as CT2. Both granulometric classes of clinoptilolite was thermally activated at 100–110 °C for 1 hour. So prepared zeolitic samples were used for the synthesis of copper forms as well as for cultivation by microoganisms.

All chemical agents used at the synthesis of modified copper forms of the natural clinoptilolite, at the analyses and preparation of nutrition medium were analytical grade (Merck and Fluka).

Preparation of Copper Forms of Natural Zeolite

Copper forms of natural clinoptilolite were prepared by the reaction of fine- and coarse thermally activated fraction of natural clinoptilolite by a reaction with $CuSO_4$ solution of two concentrations: 0.1 and 1.0 mol dm^{-3}. By this way, both copper forms, fine-grained denoted as CuCT1 and coarse-grained one, denoted as CuCT2, were obtained in consequence of an ionic-exchange mechanism. These heterogeneous mixtures were after 2 hours of stirring decanted several times and centrifuged in order to get rid of sulphate ions and then dried for 1 hour at 100 °C.

Determination of Copper Content in CuCT Samples

The copper content in the copper forms of CuCT1 and CuCT2 was determined indirectly by determination of copper content in the solution unadsorbed by the zeolitic structure, both by AAS spectroscopy at Varian Spectr AA-30, Australia and by SPECOL 11, Carl Zeiss, Jena.

The amount of Cu (II) in the zeolitic samples was determined by the energy dispersive spectroscopy (EDS) analysis using a TESLA BS 340 scanning electron microscope (TESLA ELMI a.s. with a LINK ISIS 300 microanalyser).

Contamination of the Zeolitic Material by Microorganisms

The zeolitic samples the CT1 and CT2 fractions and the copper forms were contamined in a parallel process of cultivation of microorganisms Thiobacillus ferrooxidans (TF) for different time 3,4,5 and 12 months. Before the contamination, all zeolitic samples were irradiated by a germicide lamp for sterilization. The microorganisms used were sufficiently multiplicated in a nutrition medium 9K according to Silverman and Lundgren[6]. In order to compare the surface areas and the pore volumes, a control abiotic sample (denoted as CTF) was used after application only of nutrition medium, without microorganisms.

Analysis of the Surface Areas and the Pore Volumes

The analysis of surface areas and the pore volumes of the zeolitic samples prior to the contamination and after contamination by microorganisms were realized by GEMINI 2360 (Micrometrics USA). The specific surface area was determined by low-temperature adsorption of nitrogen. Before measurements the samples were heated for 2 hours at 105 °C.

Results and Discussion

Biomodified zeolitic samples were prepared by using microorganisms in order to achieve larger sorption surface at natural zeolite of the clinoptilolite type and thus obtain more efficient results at its application in reducing the content of heavy metal residuals and other toxic materials in industrially contamined soils. Two grain- size fractions of natural clinoptilolite were studied: fine-grained (CT1) and coarse-grained (CT2). Both fractions were cultivated by microorganisms Thiobacillus ferrooxidans (TF) after thermal activation. This type of microorganism was chosen from the collection of soil microorganisms due to its accessibility as well as to the fact that some laboratory methods were available developed at the Institute of Geotechnics of the Slovak Academy of Sciences in Košice. Experimental experiences were obtained with using this type of microorganisms in the extraction of heavy metals from aluminosilicate structure. Thiobacillus ferrooxidans are applied in mineral biotechnologies for the extraction of Cu, Fe and other metals[7] and they are also present in low concentrations in the soil.

In our first studies of the biomodified forms[8] of the natural zeolite of clinoptilolite type (CT) the microorganisms Actinomycetes were used too. However, we had observed only negligibly small changes caused by these microorganisms in the studied time interval so that we did not continue in that study.

The zeolitic samples were cultivated at dynamic conditions for different time intervals: 1,3,4,5 months and 1 year. The analyses of the surface area size and pore volumes were realized also after short time intervals during the activity of the microorganisms and nutrition medium: after 3 hours and one day. The surface area and pore volumes decreased in these short time intervals.

In our previous studies[2] the content of heavy metals and PCB in the plants (spring barley-Hordeum vulgare) that were grown in contaminated soils decreased (e.g. content of Cu = 16 $mg\,kg^{-1}$, Zn = 52.4 $mg\,kg^{-1}$, PCB = 2524 $\mu g\,kg^{-1}$) compared with the plants grown in contaminated soils without an added zeolite (content of Cu = 33.1 $mg\,kg^{-1}$ Zn = 107 $mg\,kg^{-1}$, PCB = 4765 $\mu g\,kg^{-1}$). For this reason we have prepared also some model forms of the natural CT with a copper cations content, which forms could be analogically obtained in natural conditions too, if the clinoptilolite sorbs the copper cations that are in the industrially contaminated soils.

Via the contamination of the copper forms of the clinoptilolite by the mentioned microorganisms we pursued the changes of the surface, the volume of the pores and the bioleaching of copper from the zeolitic material as well.

The results of the study of the surfare areas changes of the clinoptilolite, its fine-grained CT1 and coarse-grained CT2 and its some copper forms CuCT1 and CuCT2 are in the Table I and Figs. 1. and 2. The results of the pore volumes were in a good agreement with the results of the surface

Table I
Summary of the size of the surfaces (Sa) of the fine-grain fraction CT1, coarse-grain fraction CT2 and some copper forms before and after the contamination by microorganisms *Thiobacillus* ferrooxidans (TF), and the reference sample (CTF) containing only the nutrition medium without the microorganisms

Sample	Samples contaminated with microorganisms TF	Sa [$m^2\,g^{-1}$]	Control sample CTF Sa [$m^2\,g^{-1}$]
CT1	starting sample without TF	24.2117	24.2117
	after 3 months with TF	29.635	30.6734
CT2	starting sample without TF	25.4622	25.4622
	after 3 months with TF	30.3999	28.6739
CuCT1 (0.1M)	starting sample without TF	25.1931	25.1931
	after 3 months with TF	33.0535	32.6870
	after 1 year with TF	38.2758	37.4310
CuCT2 (0.1M)	starting sample without TF	25.6817	25.6817
	after 3 months with TF	33.154	33.6086
	after 1 year with TF	42.7652	39.6286

areas. The surface areas are relatively low because they were measured only for comparison of the changes. These samples were heated only at temperature 105 °C for two hours. In our next study the surface area will be measured also after heating at the higher temperatures up to 400 °C.

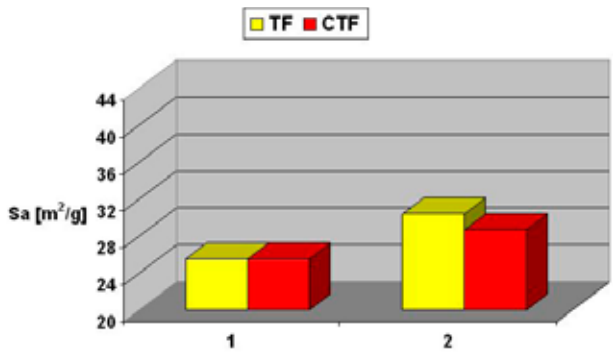

Fig. 1. **Diagram of surface area changes Sa [$m^2 g^{-1}$] of zeolitic sample CT2, *vs.* its control (CTF): 1) starting sample, 2) three months after the contamination by the *Thiobacillus ferrooxidans***

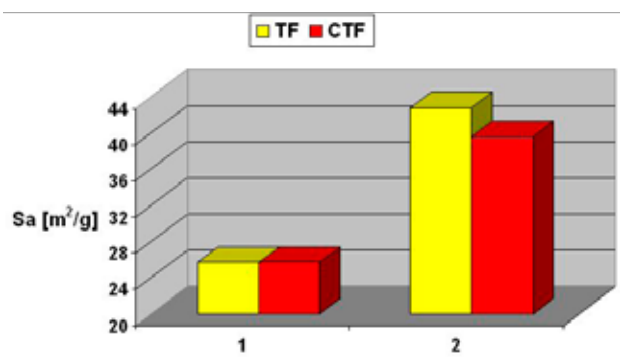

Fig. 2. **Diagram of surface area changes Sa [$m^2 g^{-1}$] of zeolitic sample CuCT2, *vs.* its control (CTF): 1) starting sample, 2) one year after the contamination by the *Thiobacillus ferrooxidans***

After the comparison of the samples before the contamination by the microorganisms TF and the reference samples CTF it was found out that even the nutrition medium itself, pH = 1.57, contributes to the enlargement of the surface area. The changes of the surface size and the pore volumes were related also with the grain size. At the fine-grain fraction CT-1 the enlargement of the surface of the reference sample (CTF) was higher compared to the sample contaminated by microorganisms. But at the coarse-grained fraction CT-2 which was contaminated by microorganisms the enlargement of the surface area was higher compared with the reference sample. However, more study is still necessary to confirm it and to find out if a change of the grain-size has not occured because such a change can cause an enlargement of the surface size.

At the copper forms CuCT the activity of the microorganisms caused enlargement of the surface area and the pore volumes at both the fine-grain and the coarse-grain fraction, compared to the reference samples.

The copper forms were studied also with respect to a copper bioleaching. According to the analyses of the copper content in the samples contaminated by microorganisms for one year their activity decreased the copper content about 60–75 % (e.g. Cu = 2.17 % in CuCT1 (0.1M) without TF; Cu = 0.58 % in CuCT1 (0.1M) with TF after 1 year). The bioleaching decreased the copper content in the copper forms of the zeolitic samples (CuCT) depending on its starting concentration and on other conditions of the bioleaching process. *Thiobacillus ferrooxidans* utilises copper ions for its metabolic processes. *T. ferrooxidans* contains in the periplasmic space the small blue copper proteins – rusticyanin, which are the principal component in the iron respiratory electron transport chain[9].

The study of the surface area changes and of the bioleaching of copper continues with the aim to obtain more detailed results after more time intervals and under various conditions.

Conclusions

The results of the analyses of the surface area size and the pores volume of the clinoptilolite and its copper forms both before and after the contamination by microorganisms Thiobacillus ferrooxidans confirmed the changes caused by the presence of the microorganisms. The results of the copper bioleaching too indicated that via the influence of the nutrition media and metabolic activity of the microorganims there occured changes of the zeolite surface and decrease of the copper content in the samples. At the present we continue in the study with the aim to obtain more experimental results about the activity of the microorganisms acting for various time intervals and at various conditions.

The combination of application of the zeolites and bioremediate technologies, which use the metabolic activity of the microorganisms looks to be a perspective ecological alternation of the contaminants degradation.

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P18 DISTRIBUTION PATTERNS OF ORGANIC POLLUTANTS IN BRNO LAKE WITH RESPECT TO ITS DEPOSITIONAL HISTORY

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Introduction

The contamination of the environment with persistent organic pollutants (POPs) has been of great concern since the 1960s. Polychlorinated biphenyls (PCBs) or organochlorine pesticides (OCPs) are found mainly in water, sediments and aquatic biota throughout the world[1,2]. Owing to an intensive agricultural and industrial production in past few decades, in particular, river and lake sediments in the Czech Republic are loaded with POPs and their levels are only partly decreasing[3]. Brno Lake is situated on the Svratka River upstream from the city of Brno and belongs to the most important water reservoirs in the SE Czech Republic. Undisturbed sediment deposits in lacustrine systems can act as environmental archive after radiodating [4,5]. Since there are only a few studies dealing with the levels of organochlorine contaminants in dated sediment cores [6–9], this pilot study is focused on the occurrence of risk elements and organic pollutants in vertical profiles of the sediment layers of the Brno Lake in order to restore the pollution history of these trace organic pollutants.

Experimental

The first sampling campaign in Brno Lake covered nine 1m-deep cores with 52 sediment samples. A 3 m-long core was drilled during the period of time when the reservoir was empty in the early 2008. The detailed sampling includes 120 samples in total; detailed sedimentological investigation and geochemistry were applied to the whole 3 m profile.

All samples were analyzed using screening methods such as magnetic susceptibility (Kf) measured on KLY-2,3 and 3S kappabridges, elemental analysis of total organic and inorganic carbon (TOC, TIC, respectively), high performance liquid chromatography (HPLC) of 16 EPA priority polycyclic aromatic hydrocarbons (PAH), and gas chromatography with flame ionization detector (GC-FID) to measure the total extractable hydrocarbons (TEH). Seven indicative PCB congeners and organochlorine pesticides were determinated by means of gas chromatograph HP 6890 equipped with μECD ^{63}Ni, cool-on-column injector and capillary column HP-5ms (60 m × 0.25 mm i.d.). From the screening results 20 samples were selected for gas chromatography coupled with mass spectrometry (GC/MS) in full scan mode to characterize the distribution of the alkylated aromatic and parent hydrocarbons and in selected ion monitoring mode (SIM) for the saturated fraction (SAT).

Results

The sedimentological visual examination together with elemental analysis of the total organic and inorganic carbon (TOC and TIC) and of the magnetic susceptibility (Kf) indicate a stratigraphic archive with roughly 5 cycles each about 60 cm thick marked by increased magnetic susceptibility, increased sand content and lowered organic carbon (Fig. 1.). These coarser grained intervals formed probably during major flooding events and migration of the lake bottom channel.

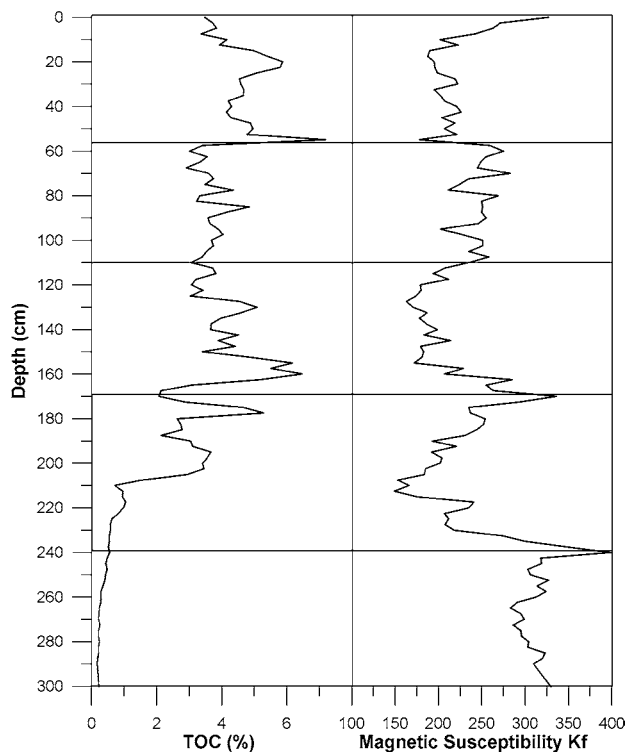

Fig. 1. **Total organic carbon and magnetic susceptibility in the deep well core profile from the Brno Lake (2008)**

The early phase of the depositional history has sedimentary structures suggesting more fluviatile environment and sandy lithology. The later phases are characteristic by increasing portion of fine-grained sediments. Laminated sediments with organic carbon of up to 7.18 % mark the highstand phases with stratified water column, which persisted over long periods of the existence of the lake. A Th-U(Ra)-K gamma-spectrometric survey shows that a probable source of these elements is in the weathered granitic material of the Brno Massif while the contribution from the U-mines is negligible.

The dating using ^{137}Cs indicates the position of the 1986 and 1961-2 events. The organic carbon, total sulphur, PAHs and PCBs provide a tentative pattern related to depositional history and flow rate. The geochemical profiles show a general upward increase in eutrophication and organic matter accumulation associated with increased cyanobacterial blooms. The sum of nine PAHs often exceeds the 10 mg kg^{-1} EPA limit value and their pyrogenic character suggests an origin in low-quality fuel and furnaces used in the villages and recreational facilities in the watershed.

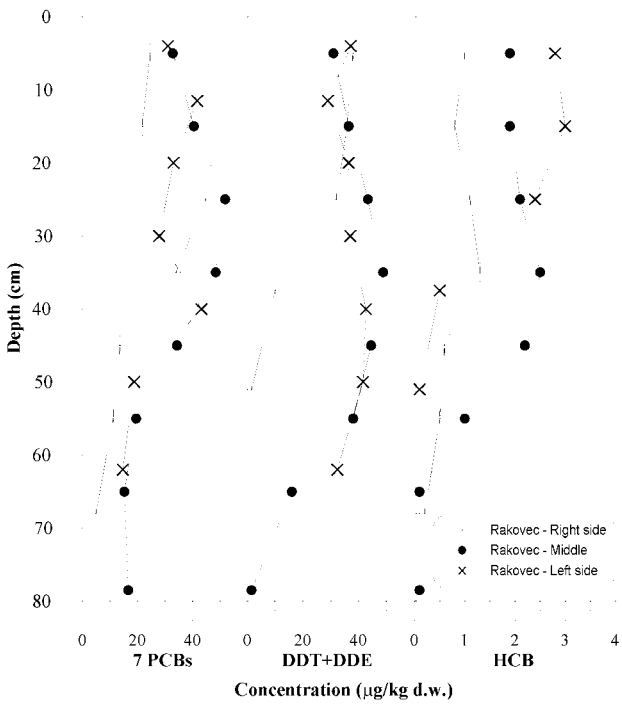

Fig. 2. **Vertical changes in organochlorine pollutants levels in one-meter-deep well core profiles from the Brno Lake (2001)**

The higher-chlorinated PCB congeners 138, 153 and 180, and *p,p*-DDE (a highly stable aerobic metabolite of the notorious insecticide *p,p*-DDT) are the most abundant organochlorine compounds detected in the sediments. PCBs show upward increase from a depth of 0.6 (0.8–0.5) m (Fig. 2.), which marks the beginning of the use of PCBs and DDT. A stagnation in the PCB and DDT concentration is observed in the upper part of the profile (0.25–0.00 m) deposited during the last decade. The differences in POP concentrations found in individual cores and layers are closely associated with variable sedimentation rates and occurrences of bottom currents affecting the organic matter deposition.

Conclusions

The pollution archive related to the depositional history of the Brno Lake suggest at least 5 cycles, each about 60 cm thick, defined by the maxima in magnetic susceptibility and minima in total organic carbon which are associated with major floods or migration of bottom currents. The amount of POPs is low in more sandy early sediments, increases at a specific depth and age throughout the basin and finally stagnates during the last decade of mud deposition indicating decreased input of POPs into the environment.

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P19 NEW RESIN GEL FOR DIFFUSIVE GRADIENTS IN THIN FILM (DGT) TECHNIQUE

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Introduction

The diffusive gradients in thin film technique (DGT) represents a relatively new approach to *in situ* determination of labile metal species in aquatic systems. The DGT device accumulates labile species *in situ* from the solution, and thus problems associated with conventional collection and filtration procedures can be eliminated. After diffusing through a layer of polyacrylamide gel of known thickness (Fig. 1.), metal species are trapped by an immobilized binding agent (usually Chelex 100) incorporated in a resin gel layer[1]. Concentration gradient established in the diffusive layer is a basis for quantitative measurement of metal concentrations in the solution. The mass of accumulated metals in the resin gel is usually determined by AAS or ICP-MS after elution of resin gel with acid.

This paper reports on the performance of a new resin gel based on alternative binding agent with 5-sulphophenyl-azo-8-hydroxychinoline groups, specific sorbent Spheron-Oxin® (ref.[2]).

Experimental

Diffusive and resin gels were based on polyacrylamide hydrogel, prepared according to the conventional procedures (DGT Research Ltd., Lancaster, UK)[1]. Chelex 100 was substituted by Spheron-Oxin® in the resin gel. DGT probe was loaded with resin gel, diffusive gel and filter on the cylindric body and sealed with a plastic top with an exposition window of 2 cm in diameter (Fig. 1.). DGT probes were deployed in the test solutions at 24 ± 1 °C.

Elution efficiency of Cd, Cu, Ni, Pb and U was obtained from subsequent elution experiments with 1 ml 1 mol dm^{-3} nitric acid. The sorption capacity of discs was estimated from accumulated mass of a metal in resin discs immersed in solutions of 25–790 mg dm^{-3} Cu. The effect of solution acidity was investigated within the range of pH 3–8. The effect of ionic strength and potentional interferents of alkali earth elemets to heavy metals was tested with model solution of sodium (2.5×10^{-5}–0.6 mol dm^{-3}) and magnesium (0–0.05 mol dm^{-3}) nitrate. The effect of competitive ligands on DGT metal uptake was investigated in solutions containing iminodiacetic acid ($0–1 \times 10^{-4}$ mol dm^{-3}) and humic substance Fluka (product No. 53680) (0–316 mg dm^{-3}).

For estimation of specific sorption capabilty to uranium, sorbents Chelex 100, Spheron-Oxin® and Spheron-Salicyl® were exposed to 5 mg dm^{-3} U solutions (pH ~ 6,7) of various carbonate concentration (0–100 mg dm^{-3}).

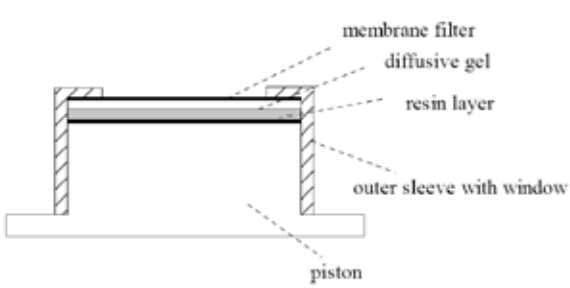

Fig. 1. **Scheme of the DGT unit**

Results

Quantitative analysis by DGT requires reproducible elution of metal ions from the resin. The elution efficiency for the Spheron-Oxin® resin gel, calculated as the ratio of the amount eluted from the gel and the amount taken up by the gel, was 0.98, 0.66, 0.86, 0.93 and 0.90 for Cd, Cu, Ni, Pb and U, respectively.

Since DGT can be used as a long-term monitoring tool, it is important to assess the sorption capacity of the resin gel. Maximum sorption capacity of 3 μmol disk^{-1} Cu was determined from the isotherm. Because the linear part of the sorption isotherm relates to aproximatelly 10 % of the capacity, discs can be deployed in natural waters for a long time period of several months.

Uptake experiments in the pH range of 3–8 showed that resin gel with Spheron-Oxin® can be used in DGT technique within the pH range of 6–8, typical for most of the natural waters.

The effect of ionic strength was investigated with solution of cadmium (40 μg dm^{-3}). Concentration of Cd measured by DGT was independent of ionic strengths up to 0.6 mol dm^{-3} $NaNO_3$.

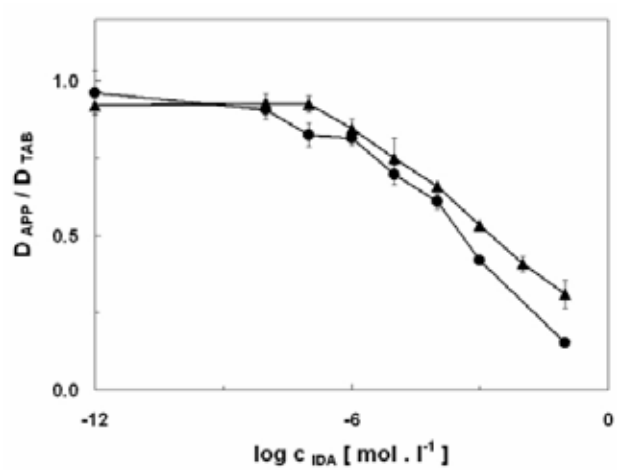

Fig. 2. **Influence of IDA concentration c_{IDA} on the normalized values of the apparent diffusion coefficients (D_{APP}/D_{TAB}) for Ni (pH ~ 7). ●Chelex 100, ▲Spheron Oxin®**

In real water systems, several other ions are present, which can compete with metals measured by DGT. The Ca and Mg are dominant cations in hard waters. Although the stability constant for magnesium is lower than for Cd, Cu, Ni and Pb, magnesium can affect DGT measured metals concentrations. Uptake of Cd, Cu, Ni and Pb was not influenced by magnesium nitrate in the whole concentration range up tu 0.05 mol dm^{-3}. New discs can be also used in high salinity waters.

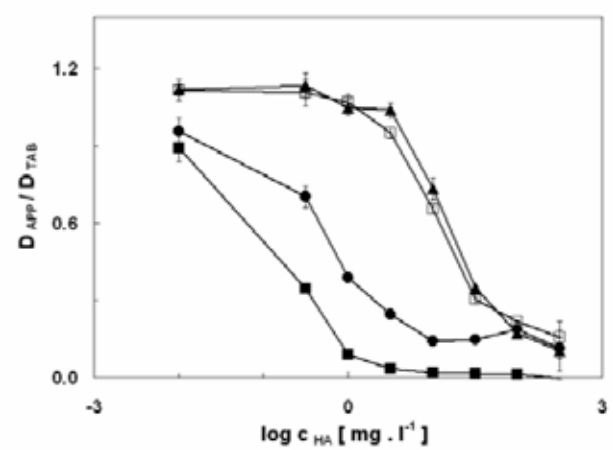

Fig. 3. **Ifluence of HA concentration c_{HA} on the normalized values of the apparent diffusion coefficients (D_{APP}/D_{TAB}) for Spheron-Oxin® resin gel (pH ~ 7). ▲Cd, ●Cu, □Ni, ■Pb**

In natural waters, variety of ligands occurs that can affect concentration measured by DGT technique. Iminodiacetic acid (IDA) was used as a model strong ligand influencing the uptake of metals. The results of deployment of DGT units, packed with Spheron-Oxin® and Chelex 100 resin gels, in Ni solutions containing IDA in the concentration range of 0–0.1 mol dm^{-3} are shown in Fig. 2.

Diffusion coefficients (D) can be calculated when taking into account diffusion area (3.14 cm^2) and thickness of the diffusive layer. Those diffusion coefficients can be considered as apparent diffusion coefficients (D_{APP}) of the metal under specific conditions. Their values represent the metal fluxes per unit deployment time, unit diffusion area, unit metal concentration in the external solution and unit thickness of the diffusive gel layer. The difference between both curves relates to stability constants of both funcional groups. This difference can be employed in speciation measurements.

Humic acids (HA) are the most widespread natural complexing ligands. Influence of the concentration of the humic substance on the D_{APP} is shown in Fig. 3., in which element-specific effect of HA can be observed. Similar results were also obtained in Refs.[3,4], in which Chelex 100 and Spheron-Thiol® resin gels were used. Due to competitive reactions,

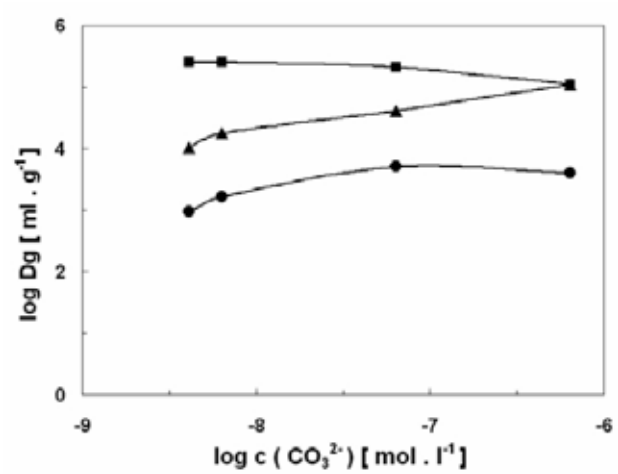

Fig. 4. **Influence of carbonate concentration on distribution coefficient of uranium for ■ Spheron-Oxin®, ▲ Spheron-Salicyl®, and ● Chelex 100 sorbents (pH ~ 6.7)**

the complex formation with HA decreases the concentration of metal species in external solution that can be measured by DGT.

Uranium, analogously to other heavy metals, forms various complexes in natural waters with a variety of ligands. Depending on pH of the solution, uranyl ions are bound in stable hydroxocomplexes and, especially carbonate complexes due to dissolved atmospheric CO_2. Influence of equilibrium carbonate concentration on distribution coefficient (D_g) of uranium is shown in Fig. 4. It is evident that the resin gel based on Spheron-Oxin® appears to be a new useful resin gel for speciation analysis of uranium in natural waters.

Conclusions

The new resin gel based on Spheron-Oxin® with anchored 5-sulphophenyl-azo-8-hydroxychinoline functional groups exhibits very strong afinity to Cd, Cu, Ni, Pb and U. In addition to conventional Chelex 100 based gels it can be applied in DGT technique and provide more information on heavy metals speciation, especially of uranium in aquatic systems.

Acknowledgement: This work was performed and supported within the Institutional research plan AV0Z40310501.

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P21 CHALLENGES IN THE ANALYSIS OF HEXABROMOCYCLODODECANE IN THE ENVIRONMENTAL SAMPLES

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Introduction

Brominated flame retardants (BFRs) are compounds widely used in commercial products to reduce and prevent the extent of fire. Hexabromocyclododecane (HBCD) is the third most produced BFR worldwide and the second most used BFR in Europe. HBCD is mainly applied as an additive flame retardant for the expanded and extruded polystyrene which are used as insulation materials in buildings and in the upholstery textile.

As the HBCD is not chemically bound to the material to which is added, it could leak into the environment through emission during a production or use of various materials, from final products or following disposal. Similarly to other BFRs, HBCD is a persistent, bioaccumulative and toxic chemical.

There are theoretically 16 HBCD diastereoisomers, however, the technical mixture consists mainly from three diastereomeric pairs of enantiomers (α-, β- and γ-HBCD), the latter one is the most abundant (75–85 %).

Both GC-MS and LC-MS are commonly used techniques for the quantitative determination of HBCD. Total-HBCD (sum of α-, β- and γ-HBCD) may be measured by GC-MS, but it does not allow the quantification of the individual isomers, because the HBCD diastereoisomers can rearrange above oven temperature of 160 ºC and also resolution of GC separation is not sufficient. The individual HBCD diastereoisomers can be determined using LC-MS/MS. On the other hand, the response in this system may be influenced by matrix ion suppression especially in the electrospray ionisation MS[1,2,3,4].

In this study, the both types of analytical systems were tested to assess the ways of HBCD determination in the environmental samples.

Experimental

The choice of environmental samples was based on: *(i)* a representativeness of both biotic and abiotic components and *(ii)* origin from Czech environment. The final extracts of tested samples including target analytes were analysed by two different chromatographic systems:

- GC-MS (using negative chemical ionization mode (NCI) with quadrupole analyzer)
- LC-MS/MS (using negative electron spray ionization (ESI–) with tandem quadrupole analyzer).

Sample Treatment

The fish muscles of species chub (*Leuciscus cephalus*) and bream (*Abramis brama*) represented biotic environmental samples. The chub and bream frequently occur in Czech rivers, are very suitable as a bioindicator of the aquatic environment contamination and were caught in the Vltava River. Also sediment and sewage sludge represented other analysed samples. The sewage sludge was collected in a sewage treatment plant (STP) and the river sediment was collected downstream from this STP, both in Hradec Králové. The individual standards of α-, β- and γ-HBCD diastereoisomers were obtained from Cambridge Isotope Laboratories (CIL, UK).

The samples (sediment and sludge after drying) were desiccated with anhydrous sodium sulphate and then extracted in a Soxhlet apparatus using a solvent mixture *n*-hexane: dichloromethane (1 : 1, v/v) for fish and DCM in case of sediment/sludge. The crude extracts were rotary-evaporated to dryness. Lipid content of fish samples was determined gravimetrically. In next analytical step, the samples were re-dissolved in 10 ml of ethylacetate: cyclohexane (1 : 1, v/v). The fat and other co-extracted compounds were removed by a gel permeation chromatography (GPC). Finally, a target fraction was rotary-evaporated and concentrated in isooctane and in acetonitrile for GC and LC analysis, respectively. GC extract was in addition treated with concentrated sulphuric acid prior the analysis.

Instrumental Determination

The target analytes were separated using either gas or liquid chromatography. An Agilent 6890N gas chromatography (GC) coupled to an Agilent 5975 Inert XL mass spectrometer (MS) was operated in NCI mode (both Agilent, USA). The system was equipped with a 15 m × 0.25 mm × 0.1 μm DB XLB capillary column (J&W Scientific, USA). The temperature of the column was programmed from 80 °C (2 min) to 325 °C at a rate of 50 °C min^{-1} and held for 5 min. Helium was used as a carrier gas at ramping flow from 1.5 ml min^{-1} (7.2 min) to 3 ml min^{-1} at a rate of 50 ml min^{-1}(ref.[2]). The ion source, quadrupole and interface temperature were 150 °C, 150 °C and 300 °C, respectively. Bromine isotopic ions $[Br]^-$ at m/z 79, 81 and molecular ions $[Br_2]$ at m/z 158 and 160 were monitored for confirmation HBCD in selected ion monitoring (SIM) mode.

A high performance liquid chromatograph carried out with a Waters Alliance 2695 HPLC instrument (USA) together with a Quattro Premier XE tandem-quadrupole mass spectrometer (Waters, USA) was operated in negative electrospray ionization (ESI–). The LC separation of the compounds was performed on a NUCLEODEX beta-PM chiral column (200 × 4 mm id, 5 μm, Macherey-Nagel), kept at 40 °C, using an (A) methanol, (B) acetonitrile and (C) deionized water gradient. The gradient was programmed as follow: 30 % (A), 30 % (B) and 40 % (C), 0–3 min linear change to 30 % (A), 60 % (B) and 10 % (C), 3–20 min kept this composition.

Specific mass transitions (m/z 640 → 79, 81) were used for a MS/MS determination.

Results and Discussion

The levels of HBCD in selected environmental samples were monitored using both types of previously described analytical systems. As mention above, HBCD consists of three diastereoisomers – α-, β-, and γ-HBCD. Fig. 1. documented the result of a GC-MS application, this technique does not enable the isomers separation, all isomers are eluted in one broaden peak. For this reason, α-isomer is used as a calibrant. Furthermore, individual diastereomers have different response factors which can cause that the less accurate results are obtained in case of higher content of β- and γ-isomers.

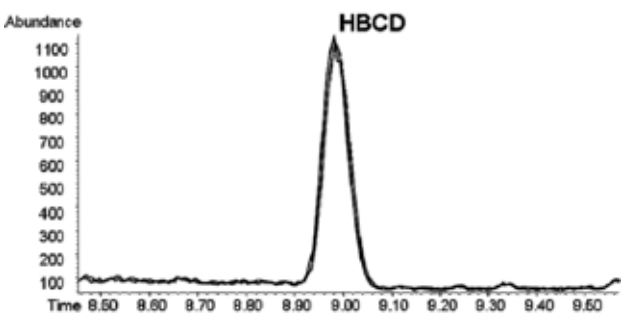

Fig. 1. **GC-MS chromatogram of HBCD (m/z 79 and 81)**

On the other hand, the LC-MS/MS allows separation of six individual enantiomers as demonstrates Fig. 2.a. The LC chromatograms show diverse patterns of the isomeric composition. In the sediment samples (abiotic matrix), as shows Fig. 2.b, γ-diastereoisomer is usually predominant, followed by α- and β-HBCD, what reflects the diastereomeric profile in various technical products. The content of the first isomer in the biota samples is the inverse and α-HBCD is the most abundant (no figure). It could be caused by biotransformation process in live organisms[5]. In addition to ability of excellent separation of individual enantiomers, LC-MS/MS provides lower limit of detection (0.5 ng g^{-1} lipid weight, 0.7 ng g^{-1} dry mass) in comparison with GC-MS system (0.8 ng g^{-1} lipid weight, 0.8 ng g^{-1} dry mass) in case of fish samples, sediment and sewage sludge, respectively.

The first results obtained from the analysis of the Czech environmental samples are resumed in Table I. The chub and bream livers contained both α-HBCD enantiomers and at the low concentration also β-enantiomers. The high level of γ-HBCD was determined in the sample of chub muscle from Podolí. This significantly higher level of γ-isomer in biota sample may indicate there is an emission source in particular locality present, because as mention above, γ-isomer dominates in technical mixture. The similar pattern of diastereomers was found also in the sediment and sewage sludge samples where only γ-HBCD was detected as shows Fig. 2.b. Comparing the results obtained within our study, it was found that the GC levels of target analytes are just a

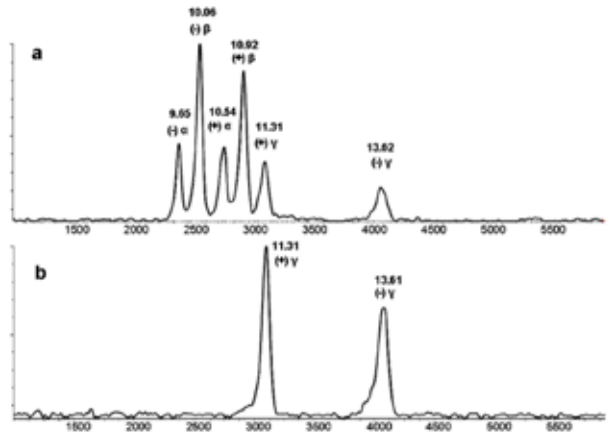

Fig. 2. **LC-MS/MS chromatogram of (a) a standard of HBCD individual enantiomers and (b) a real sample of river sediment from Hradec Králové**

slightly overevaluated by about 13 % in case of α-diatereomers. Total differences in the results between LC-MS/MS and GC-MS can be cause by usage of α-HBCD as a calibrant together with diverse response factor of individual diastereomers in GC-MS.

Table I
The results of diastereoisomers obtained by LC-MS/MS and sum of HBCD as a result of application GC-MS in fish samples (ng g^{-1} lipid weight) and sediment/sewage sludge (ng g^{-1} dry mass)

Fish	Locality	HBCD Diastereoisomers			Results of GC-MS
		α	β	γ	
Chub	Němčice	34	1.4	n.d	36
Bream	Němčice	68	0.4	n.d.	80
Chub	Klecany	6.6	n.d.	n.d.	7.5
Bream	Lysá	59	1.1	n.d.	69
Chub	Podolí	16	6	105	58
Sediment	Hradec Králové	n.d	n.d	28.1	17.7
Sewage sludge	Hradec Králové	n.d	n.d	103.9	156

Conclusions

In presented study, two different analytical techniques (GC/MS and LC-MS/MS) were tested for the analysis of HBCD isomers in environmental samples. The potential of the first system is particularly in the determination of the total HBCD occurrence in samples. On the other hand, the latter one using chiral permethylated β-cyclodextrine stationary phase allows the separation and quantification of individual diastereomers. Comparing the results obtained within our study, it was found that the GC levels of target analytes are just little overevaluated. For this reason, the need for further investigations into differences between GC and LC results are necessary.

HBCD profiles in fish are different from those observed in sediment and sewage sludge. In fish, usually α-HBCD is the major diastereomer, whereas in sediment and in sewage sludge is most abundant γ-HBCD. The processes affecting these differences in isomer composition are not fully investigated yet. LC-MS/MS is an appropriate for monitoring changes of diastereomers pattern.

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P22 THE CONTENT OF POLYBROMINATED DIPHENYL ETHERS IN FRESHWATER FISH FROM BRNO WATER RESERVOIR

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Introduction

One group of "new" persistent halogenated contaminants is represented by brominated flame-retardants (BFRs). BFRs are chemicals widely used in various products such as plastics and textiles to prevent a fire hazard. Generally two types of these compounds are in use. The *reactive* BFRs represented mainly by tetrabromobisphenol A (TBBPA) are incorporated into the polymeric materials by covalent binding, whereas the *additive* types, represented by polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs) and hexabromocyclododecane (HBCD) are embedded into a matrix of appropriate polymer.

PBDEs are similar to polychlorinated biphenyls (PCBs) in structure and characteristics such as hydrophobic and semi-volatile and/or nonvolatile. Theoretically 209 congeners (5 majorities) of PBDEs exist with specific chemical and physical properties, which lead to various biological and toxicological effects. Alike other organohalogen compounds such as PCBs, DDT and other organochlorine pesticides, PBDEs are lipophilic, very stable and resist to acids and basis, heat and light and biodegradation.

Table I
Physical and chemical characteristics of majority congeners

Congener	No. of Br atom	Log K_{ow}	Melting point [°C]	Slb. in water [mg ml^{-1}, 25°C]
47	4	6.55	83.5–84.5	0.015
99	5	7.13	90.5–94.5	0.0094
100	5	6.86	102	0.04
154	6	7.39	131–132.5	8.7×10^{-7}
209	10	9.97	302.5	4.17×10^{-9}

The concern over these anthropogenic compounds is that they can be released into the environment from products as they are not chemically bounded to the materials, and more importantly, they are persistent with a high bioaccumulation potential.

PBDEs are being found as contaminants of indoor and outdoor environments. The presence of PBDE was proved in all the components of the environment such as air, sediment and sewage sludge as well as biological samples including biota, human blood, adipose tissues and breast milk. Quite high concentrations were found in the dust in flats and offices.

Experimental

This study presents results of measuring concentration of ten PBDE congeners (BDE-3, 15, 28, 47, 99, 100, 118, 153, 154 and 183) in different species of fish using gas chromatography with electron captured detector (GC-ECD). These samples were collected from Brno water reservoir, which is situated near the Brno city.

Five head of each kind were detected and the total PBDE congener concentrations (Σ PBDEs) in the fish were amur < bream < crucian < carp.

C h e m i c a l s

PBDE standards, all with declared 99% purity, were purchased from AccuStandard, Inc. (New Haven, USA). Working standard mixtures in isooctane contained following congeners: 4-BDE (BDE 3), 4,4'-diBDE (BDE 15), 2,4,4'-triBDE (BDE 28), 2,2',4,4'-tetraBDE (BDE 47), 2,2',4,4',5-pentaBDE (BDE 99), 2,2',4,4',6-pentaBDE (BDE 100), 2,3',4,4',5-pentaBDE (BDE 118), 2,2',4,4',5,5'-hexaBDE (BDE 153), 2,2',4,4',5,6'-hexaBDE (BDE 154), 2,2',3,4,4',5',6-heptaBDE (BDE 183).

M e t h o d

A homogenized fish tissue was desiccated with mixture of sea sand and anhydrous sodium sulphate. Soxhlet extraction carried out (6 hours) with hexane/petrolether solvent mixture (96 : 4, v/v) for isolation of target analytes from the sample. Concentrated crude extracts were purified by using multi layer column (300 × 8 mm; florisil, silica, alumina). The collected fraction was evaporated to dryness and re-dissolved in 1 ml of isooctane. After addition of a few drops of sulphuric acid the organic layer was used for GC analysis. Quantification of target analytes was carried out by high-resolution gas chromatography, where two capillary columns (DB-17 MS column and HT-8 column) operated in parallel mode were used. Experiments were carried out on Agilent 6890 GC system and the analytes were identified by retention times.

Results

This paper reports PBDEs levels in freshwater fish. These were determined by gas chromatography with ECD. The identification of PBDEs in the fish samples was based on comparison of the retention times with those of the available authenticated standards. All results are summarized in

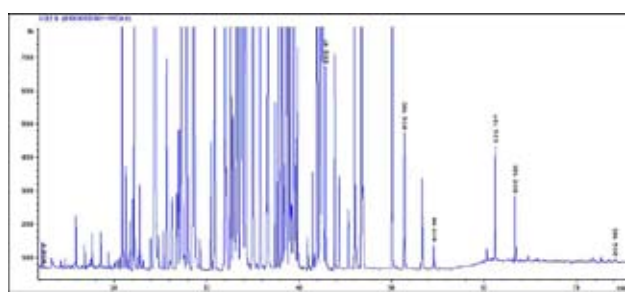

Fig. 1. **Chromatogram of carp-skin sample analysed by GC with electron captured detector in capillary column DB-17 MS**

Table II
Concentrations of PBDE detected in carp skin and muscle [$\mu g\,kg^{-1}$]

Cong./ Carp	Skin Range	Skin Mean	Muscle Range	Muscle Mean
BDE 3	21.2–26.3	24.8	14.6–17.0	15.2
BDE 15	nd*	–	nd	–
BDE 28	nd	–	nd	–
BDE 47	66.7–188	118	54.5–78.2	66.4
BDE 99	26.3–50.0	33.3	nd	–
BDE 100	49.8–136	98.2	37.9–55.1	46.3
BDE 118	nd	–	nd	–
BDE 153	42.5–77.1	52.2	nd	–
BDE 154	37.1–98.6	69.3	28.6–34.1	30.5
BDE 183	18.9–24.5	21.0	nd	–

*not detected

Table III
Concentrations of PBDE detected in crucian skin and muscle [$\mu g\,kg^{-1}$]

Cong./ Crucial	Skin Range	Skin Mean	Muscle Range	Muscle Mean
BDE 3	69.6–175	–	81.3	81.3
BDE 15	nd	–	nd	–
BDE 28	nd	–	nd	–
BDE 47	49.9–68.2	57.4	31.3	31.3
BDE 99	nd	–	16.5–27.9	24.4
BDE 100	40.0–62.3	57.8	nd	–
BDE 118	nd	–	nd	–
BDE 153	62.2	62.2	31.8–106	85.5
BDE 154	23.6–25.1	24.7	nd	–
BDE 183	nd	–	nd	–

Tables II–IV. The levels of PBDEs in amur were bello tho limits of detection, therefore they were left out in the table summary. Fig. 1. shows representative chromatogram of carp skin sample analysed in capillary column DB-17 MS with 50 % diphenyl- 50 % dimethylsiloxane stationary phase.

The most abundant congeners determined in skin of investigated species were BDE 3, BDE 47, BDE 100 and BDE 153. Greater mean levels were observed in skin then muscle tissues.

Table IV
Concentrations of PBDE detected in bream skin and muscle [$\mu g\,kg^{-1}$]

Cong./ Bream	Skin Range	Skin Mean	Muscle Range	Muscle Mean
BDE 3	42.2–62.2	48.3	26.0–31.5	29.8
BDE 15	nd	–	nd	–
BDE 28	nd	–	nd	–
BDE 47	35.2	35.2	nd	–
BDE 99	nd	–	nd	–
BDE 100	19.8	19.8	13.2	13.2
BDE 118	nd	–	nd	–
BDE 153	20.6–22.8	21.7	18.6	18.6
BDE 154	nd	–	nd	–
BDE 183	nd	–	nd	–

Polybrominated diphenyl ethers are very resistant to biodegradation and also show carcinogenic and mutagenic effects. Due to unavoidable emissions into the environment PBDEs have been found throughout the world both in abiotic (air, water, soil, sediment) and biotic (fish tissue, bird eggs, marine mammals) compartments. Into the future, will be very important and necessary monitoring these compounds in the environment and food chain especially.

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P23 EXAMINATION OF ABANDONED Sb DEPOSITS BY MINERALOGICAL METHODS IN POPROČ (SLOVAKIA)

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Introduction

The Poproč region has long been recognized as an important source of antimony in Europe. Mining began in the 17th century and finished in 20th century[1]. The abandoned Poproč Sb deposit is situated in the SE part of the Spišsko-gemerské Rudohorie Mts. (Slovakia). Geologically, the area consists of Paleozoic metamorphic rock complexes – graphitic and sericitic phyllites, metapsammites and metarhyolitic tuffs and granites. The Sb mineralization occurs as quartz-carbonate veinlets and impregnation in the host rocks. Antimony is the main ore mineral; others are pyrite, arsenopyrite, and various sulphides of Pb-Zn-Cu. The main non-metallic vein minerals are quartz, carbonates, tourmalines, albites, sericites, and chlorites. Extensive contamination related to the Sb deposit in Malé Karpaty Mts. was described previously[2,3]. The study of arsenic and antimony bindings to ferrous ochres[4,5], concentrations and distribution of As and Sb in oxidation rims of destabilized sulphides of impoundment sediment, and experimental investigation of geochemical barrier media were important. An investigation of biological and chemical oxidation was also conducted[6]. Rapid dissolution of Sb from impoundment sediment was studied at the nearby Dúbrava deposit[7]. Description of significant influence of long term mining activity and the potential effects of abandoned deposits on population health quality were described in the Zlatá Idka community (Spišsko-gemerské Rudohorie Mts.)[8]. Finally, a study of the Poproč area and its surroundings reported highly contaminated surface waters, soils, and stream sediments[9]. The As in soil samples exceeded class C standards of the Ministry of Agriculture of the Slovak Republic No. 531/1994-540 by 400 times, all stream sediment samples exceeded class C As and Sb limits, and concentrations of Sb were 100 times higher than sanitary regulatory limits for drinking water[10,11].

Experimental

Samples were collected of geological materials and natural media (groundwater, surface waters, stream sediments, soils and ochres) and selected plant species (roots and shoots) in the field area shown in Fig. 1. Samples were preferentially collected in areas near potential local contamination sources (outflow from mines, tailings ponds and theirs surroundings, and mining wastes). Samples of soils, stream sediments, impoundment material, and plant tissues were dried and homogenized under laboratory conditions by standard procedure. Special techniques were needed to selectively dissolve the solid ochre samples from the Agnes and Filip adits outflow. Selective dissolution analysis is based on the different dissolution rates of various mineral phases and compounds[12]. Based on the Fe_{OX}/Fe_{DT} ratio in analyzed ochre samples, it was possible to evaluate the relative crystallinity of the Fe^{III} oxides and oxyhydroxides. Samples were dissolved by three different methods:

- dissolution of Fe oxides and oxyhydroxides in hydrochloric acid
- dissolution of Fe oxides and oxyhydroxides in dithionate-citronate-bicarbonate (DCB)
- dissolution of Fe oxides and oxyhydroxides in ammonium acetate

Samples of soils, sediments, waters, impoundment material, ochre, and plant tissues were analyzed in the geochemical laboratories of the State Geological Survey of the Slovak Republic. Selected chemical parameters (As, Sb, Zn, Cu, Pb, Fe, Al, Mn) were analyzed by atomic absorption spectrometry in the < 0.125 mm mesh size fraction.

Results

Chemical analyses of the various environmental media sampled confirm the previous field investigation findings that the area surrounding the abandoned Poproč Sb deposit is highly polluted.

Results of selected chemical analysis data for soils are shown in Table I. The most important contaminants in the area are antimony and arsenic; their association is in accordance with the fact that the two elements are chemically similar[13,14]. Their toxicology is also similar, and they are both carcinogenic[15,16]. Extremely high concentrations of Sb were recorded in the area surrounding the Agnes adit, with values ranging from 4,700 mg kg^{-1} to 10,000 mg kg^{-1}. The highest As level measured 56,900 mg kg^{-1}. These extreme Sb and As concentrations in soils near the Agnes adit are both a result of recent contamination. A 2 m thick layer of ochre precipitates was observed over a 200 m^2 area coming from the Agnes adit until the year 2004 when stable conditions were provided. The ochre precipitate, which potentially contained similarly high concentrations of toxic elements were in the past mechanically removed by water outflowing from the Agnes adit, discharging to the catchment of the Olšava River. Chemical analysis of ochre samples collected from the Agnes adit and dissolved in HCl show high content of As and Sb in ochre material (Table II); these toxic elements have long contaminated the surface water and stream sediments of the Olšava and Bodva Rivers.

Antimony, analogous to arsenic, has an ability to bind to plant tissues and can be phytotoxic[17]. Considering the often-described mobility of these elements likely controlled by

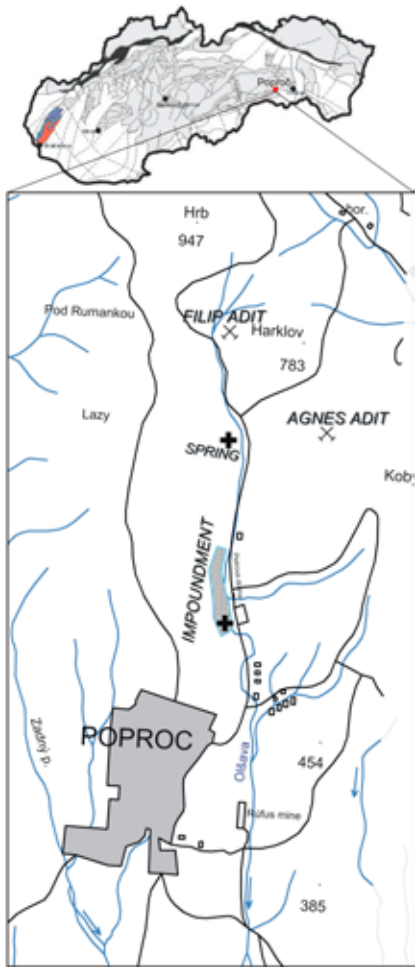

Fig. 1. **Schematic map of investigated area**

Table I
Selected chemical parameters of soil samples from surrounding of Sb deposit Poproč [mg kg^{-1}]. Fe, Mn, Al, SO4 expressed in %

	Adit Filip	Adit Filip-subs	Adit Agnes OU	Adit Agnes OA1	Adit Agnes OA2	Im-pound-ment	Im pound-ment (cover)
As	72	298	11,060	26,380	56,900	1,898	2,800
Sb	3,270	86,230	9,254	4,697	9,993	4,747	4,190
Pb	274	1723	25	<5	<5	351	352
Zn	122	352	135	1,655	3,272	71	60
Cu	54	155	48	34	52	15	13
Fe	4.23	4.29	17	30.8	35.6	0.92	5.67
Mn	0.174	0.106	0.262	0.073	0.02	<0.001	0.002
Al	7.97	6.92	4.73	1.44	0.07	5.06	3.38
SO_4	0.13	0.11	0.14	0.27	0.24	0.27	1.97

Fe, Mn oxides and pH[18], their biosorption into organisms or biovolatilization in organisms[19,20] is important. Plant tissues were sampled in the area of interest and high concentrations of As and Sb were detected in selected plant species. Near the Agnes adit, the maximum As and Sb values of plant tissue were, respectively, 45.8 mg kg^{-1} and 1.9 mg kg^{-1} (dry weight).

Table II
Ochre samples chemical analyses (mg.kg-1)

Chemical parameter	Spring 300 m NW from adit Agnes	Adit Agnes
Fe	106,000	336,000
As	8,600	34,000
Sb	5,200	6,100
Pb	104	97
Zn	160	831
Cu	44	0
Mn	1,580	210
Hg	0.4	1
Cd	0	12
Na	8,540	1,360
K	1,720	420
Ca	10,600	2,460
Mg	6,070	830
Al	7,660	2,560
S	23,300	5,950

Conclusions

The results of preliminary mineralogical and geochemical sampling near the abandoned Poproč Sb deposit reveal significant contamination of the surrounding environment. The most important toxic elements in the study area are As and Sb. High concentration levels measured in adit estuaries could be caused by inadequate mine closure/rehabilitation. Deposition of highly contaminated ochre precipitates near the Agnes adit and outflow are indicative of contaminated mine waters; inadequate recultivation has caused ochre precipitates (containing As and Sb) to be resuspended and to flow into the Olšava river catchment. The next step should be research into geochemical and mineralogical mobility and the bioavailability of the main contaminants in the environmental media.

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P24 DETERMINATION OF VOLATILE COMPOUNDS AND SACCHARIDES AT ALDER WOOD HYDROLYSIS

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Introduction

A pretreatment of lignocellulosic biomass at the production of pulp, ethanol and other technically important chemicals, is a subject of research for a long time. The pretreatment main goal is to remove lignin and hemicelluloses, to decrease celullose crystallinity and to increase porosity of the lignocellulosic material. The various physical, physico-chemical an biological processes of pretreatment are used for this purpose at present[1,2].

One method is a high-pressure water prehydrolysis. It is realized at a wide range of temperatures (160–260 °C), but in the short time intervals (0.42–7 min) in dependence on wood species[3].

The hydrothermal pretreatments of lignocellulosic materials have got a different effect on their main components. Extractives, hemicelluloses and watersoluble lignin are released from wood in the temperature range 150–230 °C. The cellulose fraction depolymerises at the higher temperatures (210–280 °C)[3,4].

At wood hydrolysis also other compounds arise except saccharides. They can be processed (methanol, acetic acid, propionic acid, 2-furaldehyde), resp. their negative environmental influence must be solved[5]. Their concentration in the hydrolysates depends on wood species and the hydrolysis conditions, mainly on the temperature and the time of the treatment.

The water prehydrolysis of biomass (by saturated water steam and hot high-pressure water) can be considered as a environmentally friendly technology, where it is not necessary to add any chemicals[6].

This paper aim was to research the release of saccharidic part and volatile compounds from alder wood (*Alnus glutinosa* (L.) GAERTN.) during the water hydrolysis.

Experimental

M a t e r i a l

Wood samples preparation

The samples from the trunk wood of 59 years old alder (*Alnus glutinosa* (L.) GAERTN.) were chipped to the dimensions 2 × 2 × 10 mm.

Wood analyses

The amount of extractives soluble in the mixture toluene-ethanol (1 : 2) was determined in accordance with ASTM Standard D 1107-96[7], the amount of cellulose by Seifert method[8] and the amount of holocelluse by Wise method[9]. Lignin amount was determined in accordance with ASTM Standard D 1106-96[10].

H y d r o l y s i s

Wood chips (2 g) were put into the stainless autoclaves with internal volume 12 cm^3 and they were refilled by distilled water. The solid/liquor ratio was 1 : 4.

The prehydrolysis was performed in the thermostate at the temperatures 160, 180 and 200 °C. The time of treatment was 30, 60, 120 and 240 min. Then the autoclave was cooled into the temperature 20 °C and the hydrolysate was filtrated.

H y d r o l y s a t e s A n a l y s e s

pH value

Hydrolysates pH values were determined by the potenciometric method with pH meter inoLab pH 720 (WTW GmbH).

Saccharides

Saccharides amount in the hydrolysates was determined in the form of aldonitrilacetates by GC method[11] at the following conditions: column – 5 % PEGA Chromaton N-AW-DMCS (0.16–0.2 mm) 240 cm × 0.35 cm, column temperature – 200 °C, injector temperature – 260 °C, detector temperature – 250 °C, detector – FID, carrier gas – N_2.

Volatile compounds

The volatile compounds in the hydrolysates (methanol, acetic acid, propionic acid, 2-furaldehyde) were determined by the method of GC[12] at the following conditions: column – Chromosorb 102 (80–100 mesh) 120 cm × 0.35 cm, column temperature – 195 °C, injector temperature – 250 °C, detector temperature – 250 °C, detector – FID, carrier gas – N_2.

Results

During the hydrothermal treatment of wood, various acid compounds are released. It is confirmed by the measured values of pH (Table I). The increase of the hydrolysates acidity causes next degradation of wood matter and the glycosidic bonds in the polysaccharides are cleavaged.

The hydrolysates acidity is due to the formation of so-called nascent organic acids (formic, acetic, propionic e.g.), which are formed by the cleavage of some functional groups from the polysaccharides and also by the decomposition of arised monosaccharides. These acids have got an important influence on the next course of the hydrolysis, therefore they catalyse the glycosidic bond cleavage in the polysaccharides[13,14].

The used alder wood contained 84.06 % of holocellulose, 41.26 % of cellulose, 21.45 % of lignin and 4.97 % of extractives.

Table I
Hydrolysates pH value

Temperature [°C]	Time [min]	pH
160	30	4.14
160	60	3.46
160	120	3.29
160	240	3.18
180	30	3.61
180	60	3.18
180	120	3.03
180	240	3.09
200	30	3.17
200	60	2.95
200	120	2.92
200	240	2.97

Table II
Yield of monosaccharides in hydrolysates

Temperature [°C]	Time [min]	Monosaccharides [g dm^{-3}]
160	30	1.56
160	60	2.84
160	120	4.46
160	240	9.12
180	30	2.46
180	60	14.40
180	120	7.96
180	240	3.15
200	30	3.05
200	60	4.71
200	120	2.23
200	240	1.92

At the temperature 160 °C the monosaccharides amount in the hydrolysates increased during the total time range (Table II). At the higher temperatures the maximum yield of monosaccharides was determined during 60 min of the hydrolysis. It is in accordance with 2-furaldehyde amount increase in the hydrolysate (Table IV). The highest concentration of monosaccharides was observed at the temperature 180 °C and the time of the hydrolysis 60 min.

The similar trend we can see at the determination of total saccharides amount (Table III) determined after the hydrolysis by 3 % sulfuric acid. At the biggest yields of the saccharides (180 °C, 60 min) approximately one half of the saccharides amount is present in the form of monosaccharides and second half in the form of oligosaccharides and polysaccharides.

In the hydrolysates there are various volatile compounds. Their amounts increase in dependence on the temperature and the time of treatment in most cases (Table IV).

Methanol, arising mainly by the methoxyl group cleavage, is extreme toxic. In some cases it is necessary to remove it before the next treatment of the hydrolysate. In hardwoods there are 3–5 % of acetyl groups (CH_3CO-), they give rise

Table III
Yield of total saccharides in hydrolysates

Temperature [°C]	Time [min]	Total saccharides [g dm^{-3}]
160	30	2.43
160	60	3.35
160	120	15.06
160	240	14.06
180	30	3.83
180	60	30.48
180	120	12.36
180	240	3.29
200	30	14.97
200	60	6.40
200	120	3.57
200	240	1.23

Table IV
Yield of volatile compounds in hydrolysates

Temperature [°C]	Time [min]	Methanol [g dm^{-3}]	Acetic acid [g dm^{-3}]	Propionic acid [g dm^{-3}]	2-Furaldehyde [g dm^{-3}]
160	30	0.11	0.28	0.10	0.02
160	60	0.31	1.35	0.53	0.14
160	120	0.63	4.46	1.17	0.63
160	240	0.54	5.77	0.94	1.64
180	30	0.18	0.67	0.18	0.05
180	60	0.47	5.35	0.92	0.89
180	120	0.82	7.73	0.74	7.24
180	240	0.84	7.52	0.65	9.23
200	30	0.29	3.41	0.61	0.46
200	60	0.40	7.72	0.59	6.43
200	120	0.97	10.03	0.66	8.17
200	240	1.13	2.95	0.82	5.12

acetic acid at the hydrolysis. The acetic acid amount increases mainly at the first 120 min of the hydrolysis, then their increase is retarded. It corresponds with the results of birch wood hydrolysis, where the increase of acetic acid amount was slowed or decreased in the hydrolysis at the temperature 200 °C[15,16].

2-Furaldehyde arises by pentose dehydratation and it can be isolated as a valuable product under certain conditions. The temperature of 180 °C causes the heavy increase of 2-furaldehyde, its concentration increases due to the condensation reactions at the temperature 200 °C.

Conclusions

From the experimental results obtained at the hydrolysis of alder wood can be concluded that at the mild conditions of hydrolysis the acetic acid and 2-furaldehyde concentration increases due to the saccharides deacetylation and dehydration, respectively. The amount of 2-furaldehyde decrease is

due to its participation in the condensation reactions at the temperature 200 °C.

The maximum of both monosaccharides and total saccharides contents were found at the temperature 180 °C and the time 60 min of the treatment.

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P26 MODELING OF DISPERSION OF WINDBORNE MATERIAL IN ATMOSPHERE

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Introduction

Air pollution modeling is an attempt to describe the functional relation between emissions and occurring concentrations and deposition. Air pollution measurements present these occurring concentrations and deposition, but they can only give a snapshot at specific locations and times. In principle, the air pollution modeling can give a more complete and consistent description, including an analysis of the causes-the emissions sources-which have led to these concentrations/deposition.

Air pollution models play an important role in science, because of their capability to investigate the importance of the relevant processes, and they play a major role in application (e.g. fire brigade intervention during chemical accidents)[1].

The atmospheric dispersion modeling, where the air pollution modeling belongs to, has a long history and it dates back to the end of 19th century, when Reynolds in 1895(ref.[2]) formulated criterion for the change from laminar to turbulent flow, in other words the diffusion, in pipes. During the next few decades, the huge progress was done in the describing of mathematical formulas and their correspondences to observations. These formulas were evaluated in analytical way and they were restricted to simple cases where the solution could be found. Nowadays, the trend of atmospheric dispersion evolution is based on the numerical solution of diffusion equation.

The diffusion equation is expressed in a form of partial differential equation (PDE). The solution of the equation can be derived by analytical process that is often very difficult to find. There exist many cases of PDE where the analytical solution to the PDE does not even exist. Thus, many kinds of numerical methods have been developed and they have been used next to the analytical ones. However, the problem exists in this kind of evaluation and it is the arising of numerical error. The only ways to check the accuracy is either to compare the results with the exact analytical solution or to compare it with the observation.

In our work we have used the first case thus the form of diffusion equation had to be simplified so as its analytical solution can be calculated. The reason originates from the fact that it is not possible to find the exact analytical solutions in the majority of cases where the solutions are looked for[2].

Mathematical Models

The general form of the diffusion equation describing atmospheric dispersion can be expressed as follows[1]:

$$\frac{\partial C}{\partial t} + \nabla C\vec{u} = \nabla\left(\mathbf{D}\nabla C\right) + \\ + chemistry + emmisions + \\ + dry\_deposition + \\ + wet\_deposition \qquad (1)$$

where C is a pollution concentration, $\vec{u}$ is a wind velocity, $\boldsymbol{D}$ are diffusion coefficients (their coordinate axes are D_x, D_y and D_z, respectively).

The chemistry term presents atmospheric chemistry term that is used for the determination of a chemical substance influence to the atmosphere and to the dispersion process itself. The emissions term expresses the rate of the emissions in the atmosphere and its relation to the atmospheric dispersion of the specific pollutant. The last two terms, dry and wet depositions, are the major sink terms in the model and besides they determine the pollutant behavior above the terrain surface.

In our case, we have used the advection-diffusion equation (A-DE) which is a part of equation (1) and where the terms chemistry, emissions and wet deposition are neglected. The reasons for this choice were that the chemistry and emissions terms are complex to find the analytical solution for them. The wet deposition plays an important role for water-soluble species only and it is not our primary concerns at this moment.

Full form of A-DE can be expressed as follows:

$$\frac{\partial C}{\partial t} = -\nabla C\vec{u} + \nabla\left(\mathbf{D}\nabla C\right) + W\frac{\partial C}{\partial z} \qquad (2)$$

where C, $\vec{u}$ and **D** symbols have the same meanings as in equation (1). W is a pollutant gravitational settling velocity.

Equation (2) can be furthermore simplified if we apply following assumptions. When the wind speed value is sufficiently large, a diffusive transport is negligible in wind direction with respect to advection (Ermak[3]). Moreover, the coefficients D_y, and D_z, depend on the downwind distance x only and they are therefore independent on the crosswind distance y and height distance z. From these facts, the diffusive terms can be simplified - the brackets are not needed anymore and the second derivatives appear. Last assumption is the stationary source with constant strength during time. Thus, the result of our simplification is a steady state form of equation (2):

$$u_x \frac{\partial C}{\partial x} = D_y(x)\frac{\partial^2 C}{\partial y^2} + D_z(x)\frac{\partial^2 C}{\partial z^2} + W\frac{\partial C}{\partial z} \qquad (3)$$

Here u_x is a wind speed in the x direction; all other variables are the same as in equation (2).

To be completed, the PDE (3) needs to have specified boundary conditions. The first condition follows from an assumption of continuous point source with constant strength located in (0,0,h) coordinates:

$$C(0,y,z) = \frac{Q}{u}\delta(y)\delta(z-H). \qquad (3a)$$

The next two conditions follow from the natural assumption that pollutant concentration approaches zero far from the source in the lateral y directions and high above the source:

$$C(x,+\infty,z) = 0\,. \qquad (3b)$$

$$C(x,-\infty,z) = 0\,. \qquad (3c)$$

$$C(x,y,+\infty) = 0\,. \qquad (3d)$$

The last boundary condition is that pollutant deposition onto the ground occurs at a rate proportional to local air concentration[3] (for simplicity, the flat ground is taken into account only):

$$\left[D_z(\infty)\frac{\partial C}{\partial z} + WC\right]_{z=0} = [vC]_{z=0}\,. \qquad (3e)$$

The deposition velocity v depends on many factors such as type and size of pollutant particles, the roughness of terrain and its other surface properties and the meteorological conditions.

A n a l y t i c a l m o d e l

The analytical solution of equation (3) with respect to boundary conditions (3a-e) is derived in (refs.[3,5,6]), and it is done by expression (4):

$$C(x,y,z) = \frac{Q}{2\pi u_x \sigma_y \sigma_z} e^{\frac{-y^2}{2\sigma_y^2}} e^{\left[\frac{-v(z-h)}{2D} - \frac{v^2\sigma_z^2}{8D^2}\right]} \times \left\{ e^{\frac{-(z-h)^2}{2\sigma_z^2}} + e^{\frac{-(z+h)^2}{2\sigma_z^2}} - \sqrt{2\pi}u_x \frac{\sigma_z}{D} e^{\left[\frac{u_x(z+h)}{D} + \frac{u_x^2}{2D^2}\right]} \times \operatorname{erfc}\left[\frac{u_x\sigma_z}{\sqrt{2}D} + \frac{z+h}{\sqrt{2}\sigma_z}\right] \right\}. \qquad (4)$$

The standard deviations of plume width and height, σ_y and σ_z, are defined in terms of their respective diffusion coefficients[3,6]:

$$\sigma_i^2(x) = \frac{2}{u_x}\int_0^x D_i(x)dx\,. \qquad (5)$$

The error function erfc (ξ) is defined in (ref.[4]) as:

$$\operatorname{erfc}(\xi) = \frac{2}{\sqrt{\pi}}\int_\xi^\infty e^{-z^2}dz\,. \qquad (6)$$

N u m e r i c a l M o d e l

In our model we have decided to use the well know method of lines, which was frequently used for solving different kinds of PDEs by classical analog and hybrid computers. In order to perform this method the given PDE (3) must be transformed into the system of ordinary differential equations (ODEs). To do that the discretization of all variables except one must be done. In our case we have discretized y (points j, step Δy) and z (points k, step Δz) variables and we have let the x variable continuous (because of the assumed wind direction).

The obtained system of ODEs is as follows:

$$\frac{dC(x,j,k)}{\partial x} = \frac{D_y(x)}{u_x}\cdot\frac{C(x,j+1,k) - 2C(x,j,k) + C(x,j-1,k)}{\Delta y^2} + \frac{D_z(x)}{u_x}\cdot\frac{C(x,j,k+1) - 2C(x,j,k) + C(x,j,k-1)}{\Delta z^2} + \frac{W}{u_x}\cdot\frac{C(x,j,k+1) - C(x,j,k-1)}{2\Delta z}\,. \qquad (7)$$

The system of ODEs (6) is solved inside the $<N_j;N_j>$ interval along the y direction, and inside the $<0;N_k>$ along the z direction, where N_j and N_k are natural numbers. Thus the total number of ODEs is $(2N_j + 1)\times(N_k + 1)$ and all equations can be solved for current time point in parallel.

The boundary condition (3a) is transformed to new initial conditions (7a):

$$C(0,0,H) = \frac{Q}{u_x\Delta y\Delta z}$$
$$C(0,j,k) = 0 \quad \text{otherwise}\,. \qquad (7a)$$

And new boundary conditions (7b–e) for the ODEs system (7) with respect to (3b–e) are:

$$C(x,+N_j,k)=0. \quad (7b)$$

$$C(x,-N_j,k)=0. \quad (7c)$$

$$C(x,j,+N_k)=0. \quad (7d)$$

$$C(x,j,0)=\frac{D_z(\infty)}{2\Delta z(v-W)+D_z(\infty)}C(x,j,2). \quad (7e)$$

For integration of the system of ODEs (7) the 4th order Runge-Kutta method was chosen. It has sufficient accuracy as is shown in the next chapter.

Results

As was mentioned above equation (3) supposes one point source that has constant strength. It means that the amount of pollutant is constant during time. The wind flows along *x* axis with constant speed and the ground is flat everywhere. In our experiments, all diffusion coefficients were constant in every space points during time, for simplicity.

Now everything is defined to solve our model of PDE (3) with boundary conditions (3a–e). The experiment has been done with following coefficient setting. The diffusion coefficients has been set like that: $D_y = 0.23\ m^2 s^{-1}$, $D_z = 0.23\ m^2 s^{-1}$ which is the parameter of ammonia, other coefficients has been: $v = 2\ m s^{-1}$, $W = 3\ m s^{-1}$, $u_x = 2\ m s^{-1}$, $Q = 0.1\ kg s^{-1}$ and $H = 1.5$ m.

The space discretization has been chosen as follows: $N_i = 600$, $N_j = 50$, $N_k = 50$, $\Delta x = 0.005$ m, $\Delta y = 0.05$ m and $\Delta z = 0.05$ m. In this case the assumed space 3 m × 2.5 m × 2.5 m has been discretized into 1,500,000 points in which the equations have been calculated.

V i s u a l i z a t i o n

We made simple program/tool for solving given A-DE and for the visualization of the results with possibility of comparison the analytical and the obtained numerical solutions. The program has possibility to make the cuts through the perpendicular grid in XY, XZ and YZ planes in any depth and the appropriate grid points can be plotted.

Fig. 1. and Fig. 2. show the XY and XZ cuts for our above-defined experiment. The level of gray (the lighter the more concentrated) expresses the amount of concentration of pollutant at each point - the most concentrated means approximately 0.5% of source concentration.

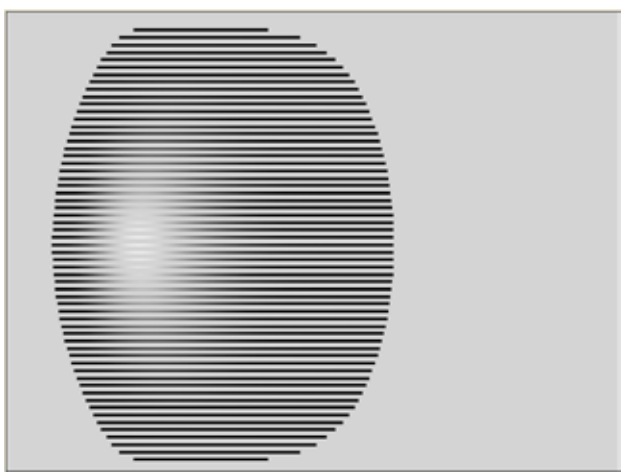

Fig. 1. **XY plane cut in zero depth, which means that the ground pollutant dispersion is shown. The wind flows from left to right**

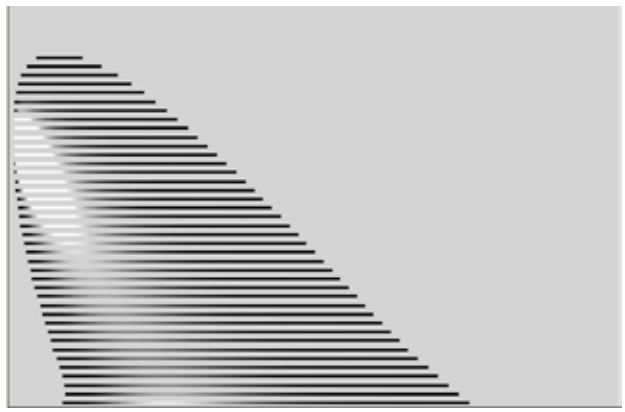

Fig. 2. **XZ plane cut in depth of 25 of overall depth 50 which means that the cut in depth of point source is shown. The wind flows from left to right**

Scales of axes (*x* is horizontal, *y* is vertical) in figures above are these:

- Fig. 1.: *x* axis: <0, 3.8> m, *y* axis: <–0.8, + 0.8> m
- Fig. 2.: *x* axis: <0, 3.3> m, *y* axis: <0, 2.5> m

The three-dimension (3D) visualization is another way to represent the calculated data in space. It has many advantages and gives to the user tool for fast investigation of the result. Many methods of gas or fluid visualization were developed however not all are suitable for our purpose. We can mention the stream ribbons, stream surfaces, particle traces, vector fields etc.[7].

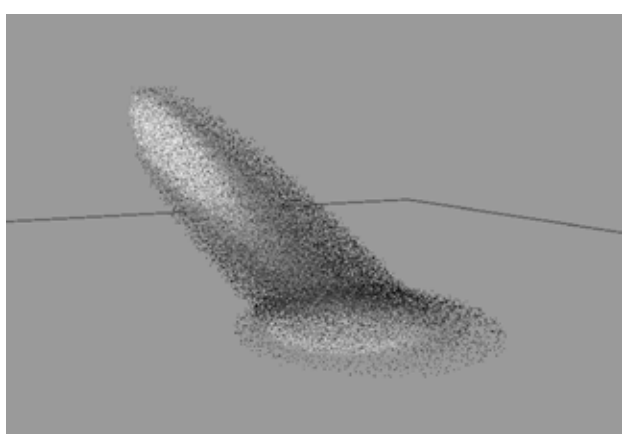

Fig. 3. **3D visualization of pollutant dispersion in the atmosphere. The white color points show the highest concentration of pollutant, the black color points show its low concentration**

In our case, we have inspired in particle visualization of pollutant concentration. After many experiments of different ways of visualization, we conclude to the one showed in Fig. 3. The concentration is expressed by two basic methods – by color and by particle count. The level of the top concentration can be adjusted – in Fig. 3. the white means approximately 1 % of source concentration, thus the user can see that this level of concentration will occurs in the presented scenario on the ground too. For the better perception of depth, the further particles are darkened, they do not interfere with the foreground particles.

A c c u r a c y

The problem of numerical calculation is the stability of the system and the accuracy of the calculation. Both depend especially on the size of calculation steps. In our case, when we have transformed the PDE (3) into the system of ODEs (7), three calculation steps exist.

The first step Δx is a step along x axis and it is used as integration step in described experiment. Thus, its size primarily influences the accuracy of obtained results.

Last two steps Δy and Δz discretize y and z variables and they have impact on the size of ODEs system (7). These steps subdivide the space in lateral directions and both are used for approximation of the second derivatives appearing in PDE (3). Therefore, they influence both the stability and the accuracy and they thus indirectly affect the size of Δx.

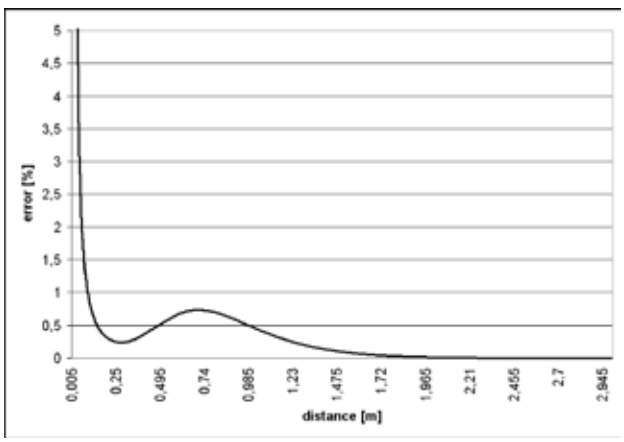

Fig. 4. **The absolute error of the numerical calculation. The vertical axis shows the error, the horizontal axis shows the distance from source along x axis (in wind direction)**

In case of our numerical solution of A-DE there exist specific properties of the calculation behavior, as you can see from our error measurement shown in Fig. 4., where the absolute error is shown. The error evolution is shown along the wind direction. More specifically the error is the mean error in all points (one plane) of the specific x distance.

The greatest error along x axis has been measured close to the source, which is caused by definition of the initial condition (7a). Fig. 4. shows other peak of error which is around 0.7 m distance from the source. That is the place where the plume reaches the ground. The boundary condition (7e) which is the approximation of boundary condition (3e) is the main reason of this error existence. It must be noted that the numerical calculations were stable in spite of measured errors.

Conclusion

The numerical method of A-DE solution has been proposed and implemented and the results have been presented. In addition, the accuracy of our model has been verified by comparison with the analytical solution.

Presented method is relatively simple and easy to implement, therefore, it can be relatively easy extend to more general form of the A-DE which will be the main goal of the future project progress. The extensions could be the general wind direction and speed, the non-flat ground with obstacles (trees, buildings etc.), non-stationary point source/sources etc. Moreover, other atmospheric parameters such as temperature and humidity and substance chemical properties will be added to the model for even more physically and chemically correct behavior.

This preliminary work will be served as a base for more sophisticated model that will be a part of the intelligent system for human protection against consequences of industrial accidents and its analysis. To do that many experiments and measurements of real substance outflows, gas dispersion etc. will have to be done.

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P27 REPROCESSING OF DANGEROUS PUT-OUT CHEMICALS AND WASTES

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Introduction

The possibilities of reprocessing of some dangerous put-out chemicals and wastes are described. The usual way for the disposal of these compounds and wastes is the effective combustion in the suitable incinerator. However, this facility must be equipped with effective furnace, vigorous supplier for air-oxygen, hold-up of waste gases for at least six seconds and also with effective cleaning of emissions[1–4]. Procedure is relatively simple, however too expensive. Some of these chemicals and wastes can be disposed by total decomposition by means of strong reactive chemical agents[5–10]. We suggested and also elaborated the chemical reprocessing of these chemicals or their wastes into some non-toxic chemicals, suitable for technical praxis.

Proposed Procedures

Benzidine (4,4'-diaminodiphenyl) (92-87-5)

Benzidine as dangerous proved chemical cancerogen is already put out from chemical praxis and usually its diposal is due to combustion in suitable mixtures of more combustible materials[1–4]. This compoun is possible to change by reprocessing into aromatic etherical compound. The base of this reprocessing is diazotation of benzidine (base, hydrochlorine, sulfate) in the methanolic solution by dry hydrochlorine and then with addition of concentrated water solution or finelly powdered alkaline nitrite in the ice bath. When diazotization is over, another methanol is added and the reaction mixture is very slowly heated (simmered) in the large flask under reflux water-cooled condenser. The diazonium salt is slowly decomposed, the nitrogen gas escape and in the methanol solution after heating up to boiling is converted into the 4,4'-dimethoxydiphenyl compound (fragrance). NOTE: The volume in the flask must be at least twice larger than volume of reaction mixture because the escape of nitrogen gas is vigorous and it is possible to reach explosion hazard of the flask. This compound often contains 4,4'-dihydroxydiphenyl as impurity, especially if this reaction is carried out in the presence of water.

When we need the pure compound, it is possible to apply alkalization by means of alkaline lye and then to use some methylating agent, such as methyl iodide or dimethyl sulfate[11,12]. In the case we use ethanol as solvent, after boiling the diphenyl compound is obtained.

The similar procedure is possible to use for other aromatic amines and diamines, such as 1,4-diaminobenzene known as p-phenylene diamine (Ursol S, alergen, harmful substance) and other aromatic diamines as 1-aminonaphtalene (alfa-naphtyl amine) and 2-aminonaphtalene (beta-naphtyl amine, Feba, Fenyl-beta, PBN), which is also very potent carcinogen!

Hydrazine (Diazane) /302-01-2/ and Also Hydrazine-Hydrate (10217-52-4)

Hydrazine in the salts (hydrochloride, sulphate) is relatively stable substance and its disposal is quite safe[5–10]. Hydrazine in the form of base or hydrate is also effective carcinogen and its disposal is usually realized by the means of combustion[1–4].This substance is possible to convert to prosperous compound by boiling with benzaldehyde resulting into substituted hydrazone, known in the cosmetic industry under trade name Benzalazine (cheap UV-absorber for suncreams).

Hydrogen Cyanide (Formonitrile, Zyklon B) (74-90-08) and Alkaline Cyanides KCN (151-50-8) and NaCN (143-33-9)

Hydrogen cyanide and alkaline cyanides are very toxic substances. They could be totally decomposed under influence of strong oxidative agents, such as alkaline hypochlorites, hydrogen peroxide and fumic nitric acid, even to nitrogen, carbon dioxide and water[13–15]. However all concentrated alkaline cyanides could be converted by heating of their ethanolic solution together with benzyl chloride and the benzyl cyanide (phenylacetonitrile). After addition of toluene into reaction mixture, followed by azeotropical distillation and removal of reaction water the phenylacetic acid ethyl ester is obtained. This compound is possible to obtain as pure substance (fragrance) after suitable drying (calcium chloride or sodium sulphate) and distillation. After reduction by hydrides it is possible to obtain 2-phenylethanol (fragrance) as the chemical substitute of natural rose oil.

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P28 APLICATION OF CHITOSAN FOR WATER TREATMENT

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Abstract

Results of experimental study focused on the removal of humic substances and turbidity by cationic biopolymer chitosan are presented. Chitosan is a natural high-molecular-weight polymer prepared from chitin, which is a polysaccharide found in the exoskeleton of shellfish like shrimps or crabs. The high content of amino groups provides very interesting heavy metals chelating properties to chitosan. Chitosan is partially soluble in diluted mineral acids such as HNO_3, HCl, H_3PO_4. We have used 0.5% solutions of chitosan diluted in 0.1M HCl. Aggregates of humic substances after inorganic coagulant or chitosan addition were separated by centrifugation. Tests were made with model humic water. The aim of this work was to found optimal use of chitosan and to compare its coagulative effectivity with that of standard coagulants – ferrous and clayey sulphate.

Introduction

Chitosan is a derivative of chitin, a polysaccharide that is the major component of the shells of crustaceans and insects. Chitin consists of long chains of acetylated D-glucosamine, that is, glucosamine with acetyl groups on the amino groups (N-acetylglucosamine). Chitosan is N-deacetylated chitin, although the deacetylation in most chitosan preparations is not complete (see Fig. 1.). Chitin itself is usually prepared from crab or shrimp shells or fungal mycelia. Treatment with an alkali then produces chitosan with about 70% deacetylation[1,2].

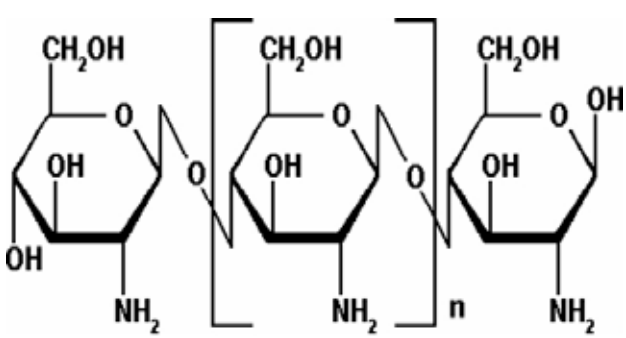

Fig. 1. **Structure of chitosan**

Chitosan as cationic polysaccharide is an important polymer flocculant in water treatment. It is known that in chitosan's molecular structure contains many amino groups ($-NH_2$) and hydroxyl groups (–OH) on the molecular chain. These –OH and $-NH_2$ groups contain single-pair electrons that can offer the electron pair to empty d-trajectories of metal ions. Chitosan can therefore be used for removal of many unwanted metal ions from water such as Al^{3+}, Zn^{2+}, Cr^{3+}, Hg^{2+}, Ag^{+}, Pb^{2+}, Ca^{2+} and Cu^{2+} etc. Because the active amino groups in chitosan molecule can be protonated with H^{+} in water into a cationic polyelectrolyte[3] the molecule shows effects of static attraction and adsorption. Thus chitosan can also flocculate particles into digger flocs which become deposited. Chitosan can be effectively used for removing COD (organic contaminant) and SS (solid suspending substances) in water treatment.

Compared with traditional chemical flocculants, chitosan has the following advantages: the required dosage is lower, a the depositing velocity is higher, also the efficiency of removing COD, SS and metal ions is better, sludge treatment is easier and there is no further pollution. Chitosan as a flocculant for treating of water will be more expensive than traditional flocculants. The objective opf our work was to prepare a cheaper composite based on the chitosan flocculant material and to make this up from lobster shells[4] and other chemical flocculants. This composite chitosan flocculant was planned not only to reduce flocculation cost but also to improve flocculating function, in comparison with single chitosan flocculant and traditional chemical flocculant polyaluminium chloride (PAC)[5].

Impurities present in the raw water are in suspended, colloidal, and dissolved form. These impurities are dissolved organic and inorganic substances, microscopic organisms, and various suspended inorganic materials. It is necessary to destabilize and bring together (coagulate) the suspended and colloidal material to form particles. Afterwards these particles are removed by filtration.

Coagulation is accomplished by the addition of ions having the opposite charge to that of the colloidal particles. Since the colloidal particles are almost always negatively charged, the ions which are added should be cations or positively charged. Typically, two major types of coagulants are added to water. These are aluminium salts and iron salts. The most common aluminium salt is aluminium sulphate, the most common iron salt is ferric sulphate. Iron and aluminium salts are used as primary coagulants and the reactions that occur after addition of these coagulants are fairly well elucidated. More recently, organic polyelectrolyte coagulants have become also used. Organic coagulants are sometimes used in combination with inorganic coagulants. Depending on the specific chemistry of the target water, polymer use can vary from as little as 5 % of the total coagulant dosage to as much as 100 % ref.[6].

Chitosan has been widely used in vastly diverse fields, ranging from waste management to food processing, medicine and biotechnology. It becomes an interesting material in pharmaceutical applications due to its biodegradability and biocompatibility, and low toxicity. The protonization of amino groups in solution makes chitosan positively char-

ged, and thereby very attractive for flocculation and different kinds of binding applications. Since most natural colloidal particles, including bacteria and macromolecules, are negatively charged, attractive electrostatic interactions may lead to flocculation[1,2,4].

Experimental

Chitosan Solutions

Chitosan is partially soluble in dilute mineral acids such as HNO_3, HCl, H_3PO_4. We have used 0.5% solutions of chitosan TM 324 (Primex, Island) diluted in 0.1M HCl. The solution was prepared fresh before each set of experiments for consistency.

Metal-Based Coagulants

Ferric sulphate [$Fe_2(SO_4)_3$] and aluminium sulphate [$Al_2(SO_4)_3 \cdot 18H_2O$], supplied by Kemifloc a.s. (Přerov, Czech Republic) or Kemwater ProChemie s.r.o., (Bakov nad Jizerou, Czech Republic) respectively were used for the experiments.

Model Humic Waters

Tests were made with model humic water. Model water was mixtures of distilled water, tap water and natural concentrate of humic substances sampled from a peatbog near Radostín. Selected model humic water parameters are given in Table I.

Turbidity was formed by addition of bentonit. Absorbance at 254 nm was measured in 1-cm cell and absorbance at 387 nm and at 820 nm were measured in 5-cm cell[7].

Coagulation Tests

Coagulation test using centrifugation as the separation method was employed. This test did allow us to study formation of NOM particles by Brownian motion (perikinetic coagulation) and has the highest possible degree of reproducibility (in any place in the world) as temperature is the only parameter influencing the kinetics of particles formation. Aggregation time was 10 and 40 minutes. Absorbance at 254 nm, 387 nm and 820 nm were evaluated.

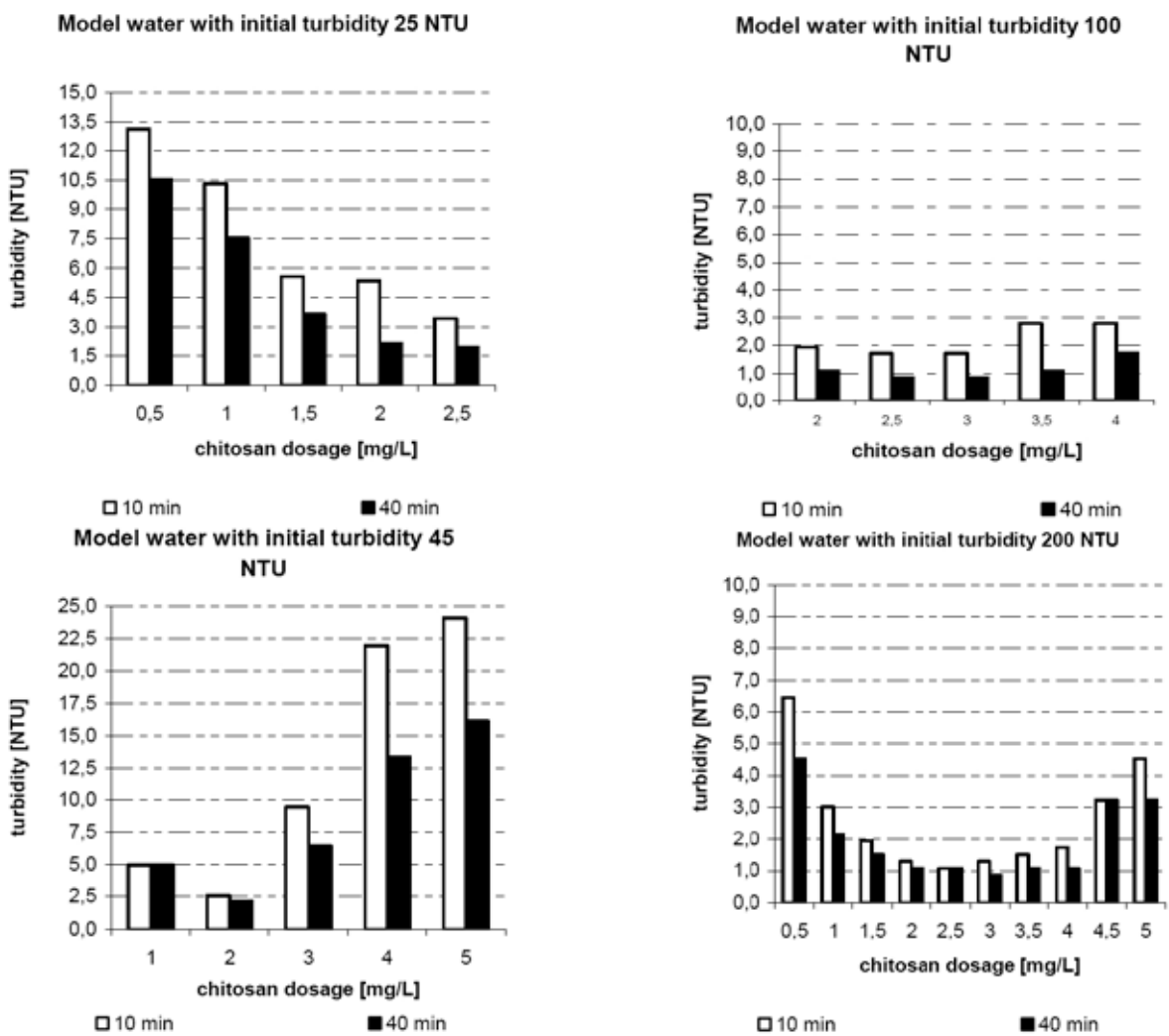

Fig. 2. **Turbidity removal comparison between different initial turbidity levels**

Table I
Parameters of starting model humic waters

pH	6.3
$ANC_{4.5}$ [mmol dm^{-3}]	0.4
χ [mS m^{-1}]	16.7
A_{254} (1 cm)	0.178
A_{387} (5 cm)	0.196
A_{820} (5 cm)	0.015
turbidity [NTU]	2.4

χ – conductivity; $ANC_{4.5}$ – acidic neutralising capacity to pH 4,5; A_{254} – absorbance at 254 nm; A_{387} – absorbance at 387 nm; A_{820} – absorbance at 820 nm

Results

Chitosan solution was dosed into model waters with values of initial turbidity 25, 45, 100 a 200 NTU. The dosages of chitosan were from 0.5 to 5.0 mg dm^{-3}.

Figs. 2 and 3. show comparison of removal of turbidity between different initial turbidity. Optimum coagulant dose ranged from 2.0 to 3.0 mg dm^{-3} of chitosan. Removal of turbidity was more than 90 % in optimum dose. Application of higher dosages induced lower efficiency of turbidity removal. Values of turbidity were lower than 2.0 NTU after treatment[8].

Conclusion

Generally, the results show very good removal of humic substances and turbidity by chitosan. The optimum pH value for chitosan coagulation was between 6–7 ref.[9].

Almost 100% removal was reached when using relatively low dosages of chitosan. Value of turbidity lower than 10 NTU was measured already at dosage of 0.5 g dm^{-3}. The results show that chitosan is a promising substitute of metal-based coagulants, which are traditionally applied in treatment of turbid humic waters.

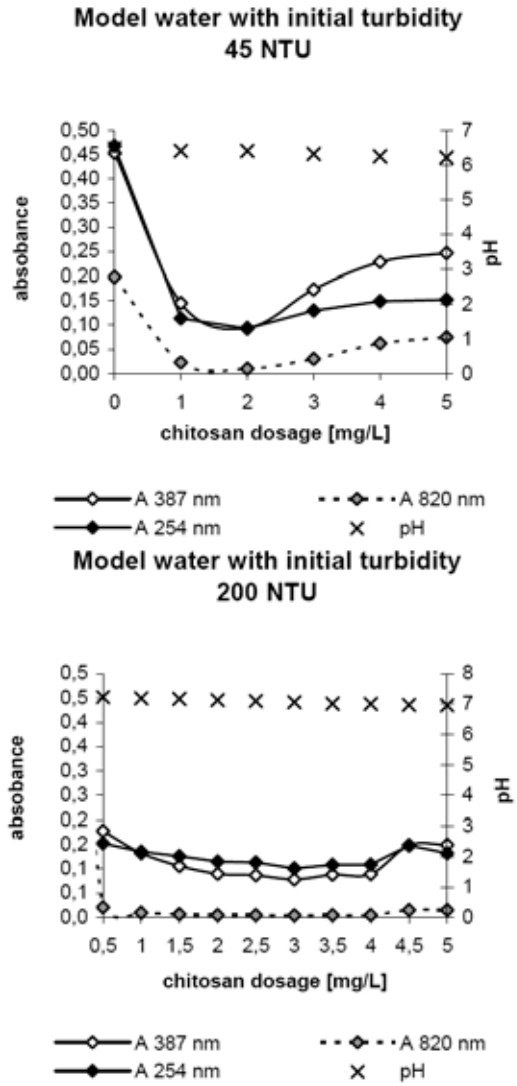

Fig. 3. **Dependence of absorbance and pH at chitosan dosage**

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P30 THE EFFECT OF WASTE BASALT WOOLS ON THE CHEMICAL, AGROCHEMICAL, PEDOLOGICAL AND HYGIENIC-TOXICOLOGICAL SOIL PARAMETERS

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Introduction

The range of soil amendments applying in agriculture of European countries, and Asia as well is considerably wider than the one in Slovakia. However, after Slovak accession to EU it has started to diversify year by year, as many soil amendments affect positively on weathering of the mineral soil elements and mineralization of organic soil elements. They reduce production of non-soluble phosphorus compounds, thereby affect positively on the level of available soil nutrients. Consequently they are alternative sustaining harvest stabilization due to the financial shortage for purchase of the industrial and organic fertilizers[1].

The trial aim was to ascertain the affect of two almost identical recycled waste rock wools produced for hydroponic plant growing (Agroban) and for building industry (Nobasyp) by the company Izomat Nová Baňa on many soil parameters.

Experimental

Characteristics of the Waste (Rock) Basalt Wools

Nobasyp is a commercial name for loose minced thermo-insulating material sold as Nobasil. It is produced as a result of milling (recycling) of Nobasil which has not met the requirements of the consumer (shape, thickness, colour, etc.). Agrodrap can be obtained by scrapping the pieces of rock wool (basalt wool) commercially sold as Agroban. In a similar way like Nobasyp, Agrodrap is produced with the aim to evaluate Agroban which is made with different parameters than the particular buyer requires. Appropriate agrochemical and hygienic-toxicological parameters of used waste rock (basalt) wools were the condition for their inclusion in biological tests.

Vegetation Trials

The pot trial was realized in a vegetation cage located at the Slovak Agricultural University in Nitra. 25 kg of Haplic Luvisol with a low content of C, accessible N and P and a high content of K were weighed out into 30 kg pots^{-1}. Every pot was sown with 100 spring barley grains. After the germination the number of the individuals was united to 75 plants per pot. There were 8 treatments repeated four times (0 – control; NS_1 – Nobasyp dose of 20 t ha^{-1}, AD_1 – Agrodrap dose of 20 t ha^{-1}; NPK – the dose of NPK fertilizers consisting of N dose – 140 kg ha^{-1}, P dose – 50 kg ha^{-1} and K – 40 kg ha^{-1}; NPK + NS_1 – fertilizers + the basic dose of Nobasyp 20 t ha^{-1}; NPK + $NS_{1/2}$ – fertilizers + half a dose of Nobasyp 10 t ha^{-1}, NPK + AD_1 – fertilizers + the basic dose of Agrodrap 20 t ha^{-1}; NPK + $AD_{1/2}$ – fertilizers + half a dose of Agrodrap). The doses of NPK nutrients (N – 3 g pot^{-1}, P – 1 g pot^{-1}, K – 2 g pot^{-1}) were calculated taking into account the N_{an} and accessible P, K contents in the Haplic Luvisol as well as the requirements of the nutrients for planned yield (6 t ha^{-1} of the grain).

Nitrogen was added in the fertilizer DAM 390, P in a form of simple superphosphate and K in a form of 60 % KCl. Nobasyp and Agrodrap doses were chosen on the basis of knowledge[2].

The spring barley harvest was performed in a growth phase DC 91. After the harvest the soil samples were taken from the whole profile of each pot where was given a research in the effect of waste rock wools on many soil parameters.

Analytical Methods

Specific electric conductivity (EC) was determined by the conductometer Hana HI 8820 N. The hydrogen ions activity was sensed potentiometrically by the glass electrode (in a soil suspension) after 24 hours of effect 1M KCl on the soil. The cation exchange capacity was detected as a sum of the base of saturation and the total acidity of soil[3]. The total carbon content was sensed oxidometrically, oxidation with $K_2Cr_2O_7$ in the environment H_2SO_4. Composition of humic substances was detected by Kononova – Beltchikova method[3]. The content of Cd, Pb, Cr, Ni was detected after mineralization with HF + $HClO_4$ by atomic absorption spectrophotometry.

Results and Discussion

The application of Nobasyp or Agrodrap (20 t ha^{-1}) has statistically significantly increased the cation exchange capacity (+ 35.7 % and + 17.2 %), the sum of exchange basic cations (+ 42 % and + 21.4 %) and the base of saturation of the soil (+ 4.7 % and + 3.6 %). The bulk density of the soil has been decreased significantly as well (–5.7 % and –2.5 %), whereas Nobasyp has determined the parameters more importantly than Agrodrap. Established results are at disagreement with the contention[4] which assert that the use of the mineral wool do not improve physical parameters of the soil. The assertion is irrational, because their experiments were narrowly focused on the effect of the wools on the water reserves increase of eroded soil.

Both materials have had a positive effect on the total carbon content (+ 2.1 % and + 9.3 %) and and the organic matter quality, thereby have increased the humic acid share in the soil, whereas Agrodrap has had more significant effect on these parameters than Nobasyp. The combined application with NPK fertilizers has increased the positive effect of the wools on the increase of the organic matter quality of the soil.

Both waste rock wools have had an alcalic effect on the soil and have deadened acidic effect of the fertilizers and the negative effect on the increase of salt content in the soil as well. They have had the same effect on the content of nine determined heavy metals in the soil (Cd, Pb, Cr, Zn, Cu, Co, Ni, Mn, Fe) as NPK fertilizers application.

At the end of the trial was the content of six heavy metals (Cd, Pb, Cr, Cu, Co and Ni) in all the treatments lower than before the start of the trial.

Conclusions

Due to the neutral effect of wools on the heavy metals content in the soil and the positive effect on the remaining soil parameters can be Nobasyp and Agrodrap regarded as soil amendments.

This work has been supported by grant projects VEGA No. 1/1346/04 and 1/4418/07

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P31 INFLUENCE OF WATER EROSION PROCESSES ON THE BOTTOM SEDIMENT QUALITY

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Introduction

Erosion processes in watersheds belong to serious ecological and economical problems because of negative consequences in terms of soil and water deterioration as well as on the environment as a whole. Sediments, detached by the erosion, bind nutrients (particularly nitrogen and phosphorus), that can significantly affect the balance of the aquatic ecology, resulting in eutrophication of lakes and rivers[1].

More studies in Slovakia have been focused on the assessment of soil erosion, based upon principles and parameters defined in the Universal Soil Loss Equation, but neither from them has dealt with nutrient transport assessment in consequence of water erosion.

This contribution deals with the nutrient transport from eroding upland fields by the small water basin Kľušov.

Experimental

Material and Methods

The study of nutrient transport assessment in consequence of water erosion has been realized in vicinity of small water basin (SWB) Kľušov situated at the Tisovec stream in the east of Slovakia. Average depth of SWB is 3.5 m and its total capacity is 72,128 m^3

According to last measuring, the quantity of sediments in reservoir caused the decreasing of SWB capacity about 33 % during 18 years. Therefore this reservoir was run the water off from 2005 to 2007 and has been chosen as model basin for our study.

For bottom sediment quality assessment, there was realized sampling of bottom sediments and also sampling of arable land in vicinity of reservoir.

Soil samples were taken according the modified methodology for nutrient transport assessment in consequence of water erosion. This methodology comes out from Decree of the Ministry of Agriculture of the Slovak Republic No. 338/2005 Coll. combine with Soil Sampling according to Mahler and Tindall[2], because Slovak decree doesn't assess soils in term of total phosphorus and nitrogen concentrations, it considers only with plant available nutrients.

Soil samples were taken from arable land in period 2006–2007.

Together with soil samples also one composite sediment sample was taken from each selected locality – along the reservoir and by the dyke.

Localities for sediment and soil sampling are shown in Fig. 1.

Fig. 1. **Sediment and soil sampling localities**

In the first stage of our research, the granularity impact of soil and sediment particles on the nutrient concentration was followed, because pollutants are preferentially attached to the finest particles (fractions below 63 microns)[3].

Samples of bottom sediments and soils were analyzed for total N and P in accredited laboratory of State Geological Institute of Dionyz Stur Spišská Nová Ves.

Results

From chemical analyses (Table I) follows that nutrient concentrations in average soil sample (P1, P2) were nearly identical with concentrations of followed compounds in fractions below 63 microns (P1', P2').

The concentrations of P, N in chosen sediment samples (S1–S8) were diverse due to irregular sediment deposition in the reservoir (Table II). These concentrations increase with proportion of the finest particle fraction and the higher concentrations are by the dyke (Fig. 2.). In this case the literary information[3] about higher concentrations of the followed compounds (N, P) in sediment samples in fraction below 63 microns has been confirmed. Because we found[4] that nutrient concentrations in sediment average sample (S1, S2, S5) and sediment fractions below 0.063 mm (S1', S2', S5') are nearly identical, in the next research we have focused only on concentration in average sediment and soil sample (Table I, II).

Table I
Concentration of N and P in the arable land

Sample	N_{tot} [%]	P_{tot} [%]
P1	0.08	0.069
P1'	0.09	0.068
P2	0.12	0.065
P2'	0.13	0.066
P3	0.18	0.082
P4	0.16	0.055
P5	0.22	0.075
P6	0.23	0.06
P7	0.20	0.077
P8	0.22	0.058

Nutrient transport assessment from soils to surface water was studied through transport of dissolved and adsorbed forms of N and P.

Table II
Concentration of N and P in SWB sediments

Sample	N_{tot} [%]	P_{tot} [%]
S1	0.26	0.112
S1' (100 %)	0.26	0.112
S2	0.24	0.113
S2' (97.65 %)	0.24	0.110
S3	0.22	0.066
S4	0.23	0.066
S5	0.24	0.112
S5' (89.24 %)	0.25	0.115
S6	0.20	0.090
S7	0.17	0.049
S8	0.14	0.070

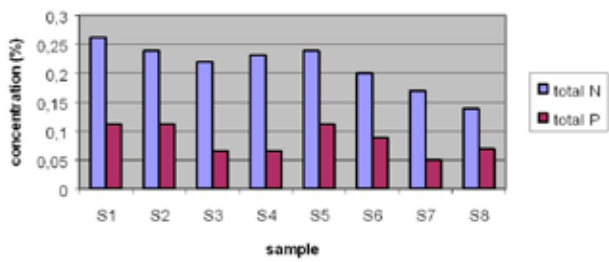

Fig. 2. **Influence of sampling distance from the dyke on the N and P sediment concentration**

For determining nutrient concentrations in dissolved phase several leaching experiments were realized. Experiment results have shown that portion of dissolved phase represented 0.46–0.65 % of total P and 0.37–0.46 % of total N in the soil sample. According these findings, dissolved phase weren't considered.

As follows from Table I, nitrogen concentrations in adsorbed phase of soils varied from 0.08 % to 0.23 % and phosphorus concentrations were in range 0.054 % to 0.082 %. It depends on rates and date of fertilizer application and also on type of grown crop and its uptake rates (samples P1–P4 before and P5–P8 after nitrogenous fertilizer application).

Conclusions

The concentration of nutrients in studied soil samples wasn't influenced by their granularity and depends on the applicable fertilizer rates and grown plant uptake.

The results from chemical analyses confirmed the literary information that the concentrations of P, N in chosen sediment samples are diverse due to irregular sediment deposition in the reservoir and increase with proportion of the finest particle fraction.Concentrations of P, N in sediments correspond to their concentrations in soils what is in accord with our results about their maximal portion in adsorbed phase.

This work has been supported by the Slovak Grant Agency for Science (Grant No. 1/0613/08).

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P32 MODELLING AND DIAGNOSING OF MECHANICAL ENGINEERING LIFE CYCLE PRODUCTION PROCESS

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Introduction

Competing business challenges to interest in production process as determined factor for quality products, environment protection, production machinery condition, required parameters for machining and elaborateness for given accuracy. One of solution is the implementation modern simulating devices and applications of analogs in production practice.

It is necessary to pay attention to production process as a determined factor for product quality, environment protection, production machinery state, required parameters for machining and elaborateness, which is given for achievement of required accuracy. One of solution is implementation of modern simulation devices and application of models in production operation.

Environmental Strategies

Environmental aspects, its loading and protection give production enterprises the possibility of evaluation of their production methodology, used technology, raw material and energy management in term of environmental influence reduction.

However, the quality of these solutions dependeds on project quality for new building-up or reconstruction of production, there is need to add ecological aspects to ordinary methods of projecting.

The ecological ones in determined term ensure sustainable development of environment. Policy, economy and ecology have got significant function. Works, function and possibilities in environmental creation and protection could be outlined in points which represent strategy of mechanical engineering development in environmental view at present:

- production of environmental suitable products,
- using of environmental acceptable technologies for their production,
- energy and raw material economy – low-waste, non-waste and recycling technologies,
- machinery and devices production for environmental protection and creation (water treatment plants, filters, separators, traps, eco-technology).

Model Creation of Mechanical Engineering Production Process

Production process is the collection of human activity, machinery and physical processes and its results are particular kinds of products. At every production process there are three factors:

- systematic activity – human work,
- work objects which are transformed to products – basic mechanical engineering products are machines, function groups, nodal points and components,
- means of work, which are production machines, devices, tools, accessories, helpers, transport and handling equipments, control technology[1].

During production process shape and composition materials are changed so that the result is the new utility value. The production process can start if production factors, inputs are disposable. One of inputs can be also the creation of production process model. That is why is necessary to have concrete data, which characterize given production process.

By analysing of process activity there are scheme where particular phases of phenomena observation or object perform these activities that are each other connected and regulated by producer's and customer's decision.

In accordance to environmental view the mechanical engineering production process is open system with its relation to surroundings. It has got full interaction among subjects. In accordance to relations this system is quite open which makes possible the full interaction to surroundings. In Fig. 1. and Fig. 2. there is the comparison of production process from the point of the view:

- influence on the environment
- renewable and unrenewable resources exploitation of raw materials and energy
- interaction with other processes
- waste production[4].

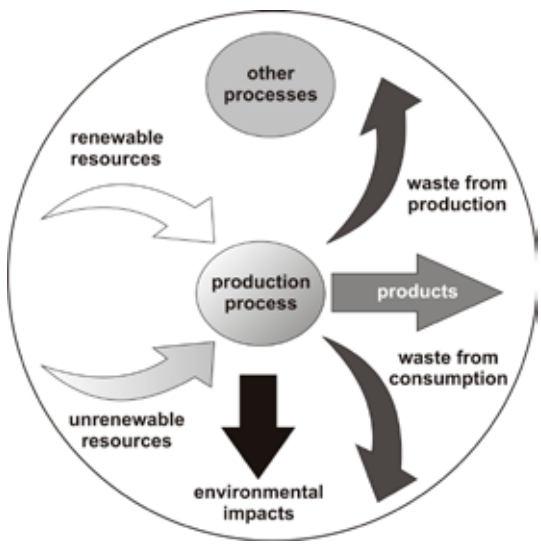

Fig. 1. **Nonenvironmental production process**

Environmental suitable production has to follow the main aim. Production process is regarded as system which creates closed structure with interaction of system environ-

Fig. 2. **Environmental production process**

ment, i.e. Closed Loop Systems, Fig. 3. This system demonstrates renewable energy, nontoxic materials in a closed loop and sustainable product design. It is rooted within circular concepts of the product life cycle.

Production process has also got its life cycle. There are characterized its production, system, technological and environmental phases. According to production aspects the process can be in beginning phase of its cycle. However according to environmental impact view can be out-of-date or according to qualitative parameters in leading countries "outsider". Working out of equivalent model of production process is has got several phases as followings:

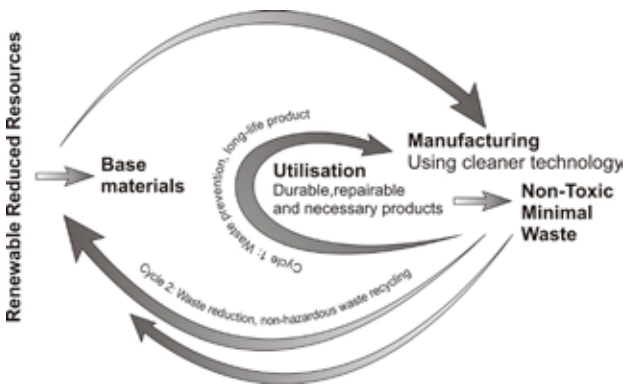

Fig. 3. **Closed Loop Systems**[4]

P r e d i c t i o n A n a l y s i s

Estimation of production running, its inputs and outputs is possible to make or by considering that production has got the same way eventually similar way or quite new and different way. The way is determined as kind of application, its evaluation, modification or change of production, systemic, technological and environmental approaches. It is important to establish the reliable obtained information. It can reach to use accurate methods of data collecting or application of measurement methods if there is an existing operation plant where it is possible to obtained parameters.

D e v e l o p m e n t o f M e t h o d

According to number of inputs and outputs, we can choose strategy or methods for model development. We obtained various types of models with the combination of individual parameters. If we choose the main point as economical point and inputs will be reduced only gains, expenses, costs per hour, costs per piece, etc. Very simply model comes into existence but in real operation there is not applied. Many customers ask for quality, ecology, and modern technology not only low price.

M o d e l I m p l e m e n t a t i o n

There is a verification of prediction and correctness of used methods. If the preparation is worked out in details then the implementation is simple and the model can be applied in short time. Implementation makes possible to modify a model and it creates other opportunity to regulate and to adjust the model.

C o n t r o l l i n g o f M o d e l

After successful implementation of model there is final period – i.e. control. It is the adjusting according to external and internal operational demands. All feedback relations in system can be used and following modifications, which do not influence the model creation but only they simulate all changes in production.

A p p l i c a t i o n o f M o d e l

Model, which is created in this way, exists from its beginning to its end and this model makes possible to eliminate or minimize mistakes. These mistakes would be developed directly in operation without the using of previous simulation.

Model becomes the control tool for production process. Mistakes in model are cheaper than real production and changes, which are done in model, are reversible as well. Production enterprises can use Model for improving or representation for customers, contact persons from state institutions, for certification of quality of environmental management, for training and courses of employees or for comparison of product quality to competing product. In model there are all accessible methods and technologies, which product operator requires. One of methods is Life Cycle Assessment (LCA)[2].

Life Cycle Assessment

Life Cycle Assessment is very often term predominately in environmental management field. Every mechanical engineering product shows environmental "track" in environment and this way these environmental impact are evaluated during all its life cycle – from the cradle to grave. It is complicated to obtain enough data number from operation. Or obtained data are different values, units or time and accuracy of obtaining.

Production process is also concerned hundreds of products and its characters are complicated. In LCA there are assessed for example harmful substances released into the air and water, amount of solid waste as well, energy and raw material consumption, human environmental effects, who are in production. Tools of LCA cover general matters as well as localizations independent from evaluation methods.

Modelling of process means the using of all available methods from LCA, SEM, standards ISO 14000 and 9000 and technological processes, LCA technologies and likewise. Complex model can also simulate production accidents[3].

Multicriterial Environmental Evaluation of Using Chemicals in Individual Plants

This evaluation is based on quantification of parameters for environment quality by means of unbalanced evaluating effects of production process.

multicriterial evaluation for using of chemicals in production process								
	1.	2.	3.	4.	5.	6.	7.	
	waste chemical amount	amount of air pollution	employees with hypersensitiveness	amount oils and process liquids	water consumption in thousands	number of made products	electrical energy consumption	
units	[ton/year]	[mg/m³]	[person]	[liters]	[m³/year]	[pieces]	[MW/year]	
polarization influence for environmental load [v-]	+	+	+	+	+	-	+	
	values of criteria for individual operational plants							
individual operational plants 1	a_{11}	a_{12}	a_{13}	a_{14}	a_{15}	a_{16}	a_{17}	Q_1
2	a_{21}	a_{22}	a_{23}	a_{24}	a_{25}	a_{26}	a_{27}	Q_2
3	a_{31}	a_{32}	a_{33}	a_{34}	a_{35}	a_{36}	a_{37}	Q_3
4	a_{41}	a_{42}	a_{43}	a_{44}	a_{45}	a_{46}	a_{47}	Q_4
5	a_{51}	a_{52}	a_{53}	a_{54}	a_{55}	a_{56}	a_{57}	Q_5
the smallest value of criteria	n_1	n_2	n_3	n_4	n_5	n_6	n_7	
arithmetical average of criteria	$\bar{a}_1$	$\bar{a}_2$	$\bar{a}_3$	$\bar{a}_4$	$\bar{a}_5$	$\bar{a}_6$	$\bar{a}_7$	
standard deviations	s_1	s_2	s_3	s_4	s_5	s_6	s_7	
coefficients of correlation	$r_{11}=0$	$r_{21}=r_{12}$	$r_{31}=r_{13}$	$r_{41}=r_{14}$	$r_{51}=r_{15}$	$r_{61}=r_{16}$	$r_{71}=r_{17}$	
	r_{12}	$r_{22}=0$	$r_{32}=r_{23}$	$r_{42}=r_{24}$	$r_{52}=r_{25}$	$r_{62}=r_{26}$	$r_{72}=r_{27}$	
	r_{13}	r_{23}	$r_{33}=0$	$r_{43}=r_{34}$	$r_{53}=r_{35}$	$r_{63}=r_{35}$	$r_{73}=r_{37}$	
	r_{14}	r_{24}	r_{34}	$r_{44}=0$	$r_{54}=r_{45}$	$r_{64}=r_{45}$	$r_{74}=r_{47}$	
	r_{15}	r_{25}	r_{35}	r_{45}	$r_{55}=0$	$r_{65}=r_{55}$	$r_{75}=r_{57}$	
	r_{16}	r_{26}	r_{36}	r_{46}	r_{56}	$r_{66}=0$	$r_{76}=r_{67}$	
	r_{17}	r_{27}	r_{37}	r_{47}	r_{57}	r_{67}	$r_{77}=0$	
constants of reduction	k_1	k_2	k_3	k_4	k_5	k_6	k_7	

Fig. 4. **Multicriterial evaluation for using of chemicals in production process**

Objectivity of solution has got number of evaluating coefficients, their optimal number in interval 20–25. Deriving from following relations it is possible to determine Q_j – value of environmental chemical load:

$$Q_j = \sum_j \frac{a_{ij} - \bar{a}_j}{s_j} \times k_j , \qquad (1)$$

where:

Q_j – value of environmental load,
a_{ij} – value j-element of vector,
$\bar{a}_j$ – average value of j-element
s_j – standard deviation of modified j-descriptor,
k_j – reduction constants.

Example for application of multicriterial evaluation for using of chemicals in production process is in Fig. 4.

It is possible to determine from application of multicriterial evaluation which operational plant is the most loaded, i.e. the plant with the higher Q_j- value.

Conclusions

Methods for monitoring and checking of technological, chemical and environmental aspects for production can have other applications as well.

There are in various enterprises for development of technology or optimalization of operation according to customer's, producer's or environmental engineer's demands. This period is period IT technologies and it could show that environmental pollution is slowing because operation plats of individual productions began to behave more understanding.

Model of life cycle for production process is one of tools that helps producers made with optimal technology, develop products with optimal balanced performance and costs, characters which are up to standards for environment, health and safety.

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P33 SIMULATION OF CHEMICAL FACTORS IN WORKING ENVIRONMENT

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Introduction

Mechanical engineering has not got so many environmental impacts as chemical industry but its technologies often use various chemicals to improve its operations. Working environment of mechanical engineering is also a subsystem of global geosphere environment.

People spend so long term that they identify this working environment with their microenvironment. People are often influenced by several factors in this "small world" of them. These factors categorize some working activities from point of view the environmental and health risks. The categorization of our working activities involves the known details about factors of work and working environment.

At industry plants there is most of workers exposed to a combination of multiple factors of working environment which influence their health and comfort. The category is determined separately for individual factors but there is no methodology of working activities assessment for any combination of individual factors[1].

By means of modern software simulation there is designed the methodology with the look-up functions of program Vensim for a complex assessment of working environment in specific conditions of mechanical engineering enterprises.

Chemical Parameters in Cutting Process

In cutting processes there are a lot of chemical parameters which improve or make worse human working environment. These chemicals exist in various form and states. The liquids and aerosols create predominant part of the states.

Cutting Fluids

Cutting emulsion is a dispersion system of two insoluble liquids. One creates microscopical drops dispersed in the other liquid. At machining only emulsion type: *oil in water* is used. Milk appearance or transparent appearance depends on size of oil drops. For the first case the drops are larger and for the second case the drops are smaller than 10 mm[3].

Mineral oil and water almost do not generate an emulsion. That is why the other component is needed – emulator which covers oil drops with absorption coat protecting their repeated mergence. Soaps, organic amino compounds, sulphonate compounds and the like are used as emulators.

Mineral oils conditioned with emulators are called emulsive oils. They also contain anticorrosive, antibacterial and high-pressure additives. The content of mineral oils in emulsive oils is 50–75 %.

The second component of basic emulsion compounds is water which has got very good cooling effect. However, raw water has got a lot of defaults for a preparation of industrial emulsions, i.e. presence of limestone and magnesium which are liquated and they create hard-removable sediments on metal surfaces. The sediments silt up piping, sieves, tanks and also seal the functional parts of machinery[2].

As well the corrosive influences and high contents of micro-organisms are harmful in water for an emulsion preparation. An anticorrosive protection is performed by the anticorrosive additives, i.e. soda, borax, sodium phosphate or sodium silicate. These additives soft water and decrease its surface tension. They improve wettability, cooling effects and create alkaline environment. Alkaline environment is suitable from point of view an anticorrosive protection of ferrous metals. There are pH under 9.0 because safety and health reasons. If pH is higher then there is a damage of protective coats and rubber parts of machines.

The main default is an inclination to micro-organism contamination what makes short cutting fluid durability by decisive way. If there is an emulsion value of pH 9.5 and higher then micro-organism development is decelerated. However, in this condition the emulsion is health unsuitable, irritate skin and airways.

Additive BIOSTAT® for Cutting Fluids

BIOSTAT® is a common name for new generation set of antibacterial and fungicidal preparations which have been developed for 8 years in the framework of environment friendly production.

Preparation set BIOSTAT® is inorganic origin and its influence is based on three activity principles:

- the strong electrochemical influence of Ag ion operating directly with energy reaction in an electron-transportation string of cellular proteins
- the irreversible denaturation of sulfhydryl groups of micro-organism proteins by means of the ion exchange H by Ag
- the strong oxidation possibility of product of BIOSTAT®, which causes the catalytic oxidation H_2O molecule and air oxygen generating hydrogen peroxide. Hydrogen peroxide is fissioned consequently into H_2O molecule and oxygen radical[3].

Determination of Parameters for Model

Simulation software Vensim is a graphical model tool. By means of it, we can draft, document, simulate, analyse and optimize various simulation models of industrial technologies. One of the technologies is also machining with using of chemicals, which belongs to the difficult tasks solved in environmental engineering protection.

The keystone of s model imulation is determination and application of following variables in the sketch with nodes and connecting curves:

- level parameters – amounts of cutting fluids
- rates – operations or technological processes
- constant variable – chemical factors
- auxiliary variables – derived parameters for chemical and environmental impacts.

Model for Cutting Fluid Chemicals

In Fig. 1. there is the sketch of applied parameters for using of cutting liquids in machining and in Fig. 2. there is the simulation of these parameters.

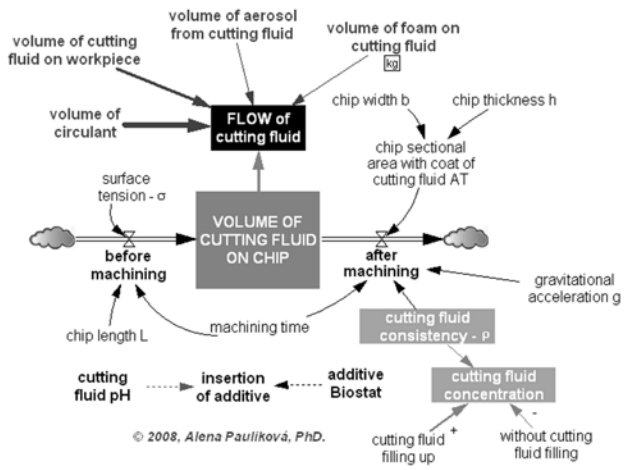

Fig. 1. **Used parameters of cutting liquids in machining**

Simulation for Cutting Fluid Chemicals

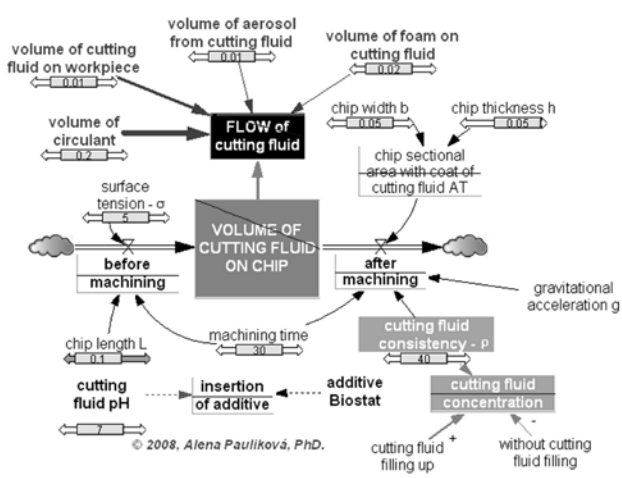

Fig. 2. **Simulation parameters of cutting liquids**

There is a chemical world of systems driven by cause and affect. Those systems include production, protection, biological, chemical, thermodynamic or workflow.

Environmental or chemical systems can be modelled as nodes representing system variables and connecting lines representing causal effects. The changing value of one variable can cause another to increase or decrease. Understanding how a system really works is the first step towards using, improving or automating[4].

Results

We can change chemical status of the overall machining process with the movement of slider. Every constant has got its own slider. The change of constant value influences the value of auxiliary variables and then they influence value of level parameters.

This similar model can help the technicians or workers to properly dose various additives, to fill up fresh cutting fluids, to increase total productivity and to save expenses.

Conclusions

By the assessment of individual quantitative indicators of cutting fluid chemical status we can draw these conclusions:

- used cutting fluids began to spoil the most often by long term of cleaning of individual machines and cutting fluid reservoirs
- the insertion of additive BIOSTAT® into cutting fluids to lengthen their durability and cleaning intervals.
- the declination of Ag is caused its sedimentation of undissolved additive in the cutting fluid reservoirs and its removal by means of workflows or waste-flows of cutting fluids
- the cutting fluids flows need for their cycle the segment of sedimentation reservoir and filters to improve working environment
- additive BIOSTAT® does not cause allergic reaction of respiratory or dermal characters.

This work has been supported by VEGA 1/3231/06: (2006–2008) Modelling of Working Environment Factors and Their Optimalization in Specified Conditions of Mechanical Engineering Enterprises.

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P34 MULTI-RESIDUE METHOD FOR THE ANALYSIS OF PESTICIDES AND MYCOTOXINS IN CEREALS BY LC-MS/MS

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Introduction

In the recent decade, liquid chromatography–tandem mass spectrometry (LC MS/MS) operated in a selected reaction monitoring mode, has become the main tool for the analysis of food and environmental contaminants presenting wide range of physico-chemical properties. This approach allowed the introduction of multiresidue methods with up hundreds target analytes determined in a single run. However, most of these methods are focused only on one group of food contaminants such as pesticides, veterinary drugs, mycotoxins, plant toxins, etc. Considering the fact, that sample preparation/detection principles are basically similar for most of these methods, we attempted to determine pesticide residues and mycotoxins - contaminants representing different sources of origin in single run. The comprehensive LC-MS-MS multi-residue method has been developed and validated for 14 *Fusarium* toxins, 4 aflatoxins, ochratoxin A, 3 *Alternaria* toxins and 200 pesticides.

Experimental

Chemicals and Reagents

Certified pesticide and mycotoxins standards were purchased from Dr. Ehrenstorfer GmbH (Germany), Riedel de Haen (Germany) and/or Biopure (Austria). Individual analyte stock solutions (concentrations in the range 0.3–3mg ml^{-1}) were prepared in either methanol or acetonitrile, depending on the solubility of particular analyte. These solutions were used for preparation of mixed standard solution in acetonitrile (10 μg ml) and stored in –18 °C. Deionized water for preparation of a mobile phase was produced by Milli–Q apparatus (Millipore, Germany). Ammonium formate for mass spectrometry was obtained from Fluka (Buchs, Germany). Acetonitrile (Sigma-Aldrich, Germany) and methanol (Merck, Germany) were HPLC gradient grade solvents for pesticide residue analysis.

Sample Preparation

5 g of sample were weighted into 50 ml PTFE centrifugation tube. Then, 20 ml of extraction mixture of acetonitrile/water/formic acid (75 : 24 : 1, v/v/v) were added and the tube was placed onto laboratory shaker for 90 min. After this time, the tube was centrifuged (Hettich, Germany) at 11,000 rpm for 5 min and aliquot of extract was diluted by a water in a ratio 2 : 1 and filtered through a 0.45 μm PTFE filter IsoDiscTM (Supelco, USA), and transferred into a vial.

HPLC-MS-MS Analysis

The HPLC analyses of selected pesticides and mycotoxins were performed using an Alliance LC system (Waters, USA) equipped with an Atlantis T3 column (100 × 2.1 mm I.D., 3 μm particle size, Waters, USA) maintained at 30 °C. The mobile phase contained 0.005M ammonium formate in deionized water (A) and methanol (B), flow rate was 0.3 ml min^{-1}. The optimized chromatographic method started at mobile phase composition of 5 % of B and was hold for 0.5 min, then rising linearly to 60 % of B and then 100 % at 15 min. This composition was held for 8 min to remove co-extracted matrix from column, 6min re-equilibration to initial mobile phase composition followed. Sample injection volume 8μl was used in all experiments.

HPLC system was connected to tandem mass spectrometer Quattro Premier XE (Waters, USA) operated in positive electrospray ionization mode. The capillary voltage was set to 3,500 V, source temperature was maintained at 120 °C and desolvation temperature was 380 °C. The masses of parent and daughter ion, cone voltage and collision energy were tuned previously for each analyte and two MS/MS transitions were acquired for each of them.

Results

Optimization of Sample Preparation

QuEChERS extraction method published and extensively tested in a recent years[1–3] has become the widely used method for isolation pesticide residues from various matrices. QuEChERS method employs acetonitrile (MeCN) extraction followed by partition induced by added salts. If necessary dispersive SPE clean-up is performed. The partition step discriminate a lot of bulk matrix compounds such as sugars and/or acids, which would interfere with determinative step, however also recovery of polar target analytes is reduced. For this reason, the QuEChERS has not become an extraction method of choice for mycotoxins, especially for relatively polar B trichothecenes.

It should be noted, that existing multi-mycotoxins methods are based on the extraction by MeCN i.e. use similar solvent as QuEChERS. Although the sources of food contamination by pesticides and mycotoxins are fairly different in their nature, from analytical point of view there are a large number of representatives of these two groups, possessing similar physico-chemical properties. On this account, it is a conscionable concept to put together analysis of mycotoxins and pesticide residues in one multi-toxin method.

The bottleneck of such method might be the sample preparation step, since it is necessary to achieve acceptable recovery for all analytes and at the same time discriminate extraction of matrix components, which could cause suppression of ionization as far as electrospray (ES) is employed in LC-MS method. In principle, achieving simultaneously these two requirements is impossible, and, therefore the only feasible approach is to examine extract directly without any purification[4–5].

Several extraction solvents including MeCN, methanol (MeOH) and their mixtures with water were tested to achieve good recovery for a wide range of analyte polarities. MeOH and its mixtures with water offered good extraction efficiency for polar and semi-polar compounds, however high amount of matrix was also co extracted. Only aqueous MeCN was used, because of poor extraction of polar analytes by pure solvent. It is a common practice in many multi-mycotoxin methods to employ azeotropic mixture of 84 % MeCN and 16 % water. This approach was developed for easy purification by Mycosep and subsequently solvent evaporation, nevertheless omitting purification step enabled improvement of extraction efficiency of polar compounds (such as DON-3-glucoside, acephate, propamocarb) by increasing of water content in extraction mixture up to 25 % (v/v). At the same time, acceptable recoveries were still obtained for relatively non-polar analytes represented e.g. by zearalenon and pyrethroid insecticides.

It should be noted that the addition of water prior to extraction into low moisture samples as cereals is also recommended by a document N° SANCO/2007/3131. To improve recovery of fumonisin mycotoxins and protect unstable base-sensitive pesticides against the hydrolysis, addition of 1 % of formic acid into extract mixture was necessary.

The final extract was diluted by water to decrease content of MeCN and consequently to reduce matrix effects. Different ratios of MeCN and water in a vial were tested, nevertheless the content of water higher than 50% caused precipitation of matrix components and consequently decreasing of recovery of non-polar analytes.

Optimization of LC-MS-MS

As mentioned above, no purification step was employed, so chromatographic separation plays an important role to separate analytes and matrix to decrease matrix effects. Although slow gradient was used, very strong signal suppression (over 80 % lower signal of matrix matched standard as compared to solvent standard) still occurred for some of later eluting compounds.

Many LC-MS pesticide multi-residues method use only ESI^+ ionization, because almost of analytes ionize only in positive mode or better sensitivity/selectivity is achieved under these conditions. Although B-trichothecenes offer better sensitivity in a negative mode by formation adducts with acetic or formic anion (depends on composition of mobile phase), ionization in positive mode is also possible. The main advantage of positive mode is a compatibility with more than 95 % pesticides and other mycotoxins (aflatoxins, fumonisins) which do not give any ions in ESI^-.

Method Validation

The distribution of recoveries of all analytes in wheat is showen in Fig. 1. The recovery and repeatability of particular analyte was obtained from analysis of spiked wheat material at 100 $\mu g\,kg^{-1}$. The extraction method offers a good recovery for both, acids and bases and covered a wide range of polarities. The trueness of method was demonstrated by analysis of reference materials for mycotoxins (ochratoxin A, deoxynivalenol, nivalenol, T-2 and HT-2 toxins) and proficiency testing materials obtained from FAPAS 0950 and EUPT-C1/SRM2. For all reference materials and positive findings in proficiency tests were achieved satisfactory score $|z| < 2$.

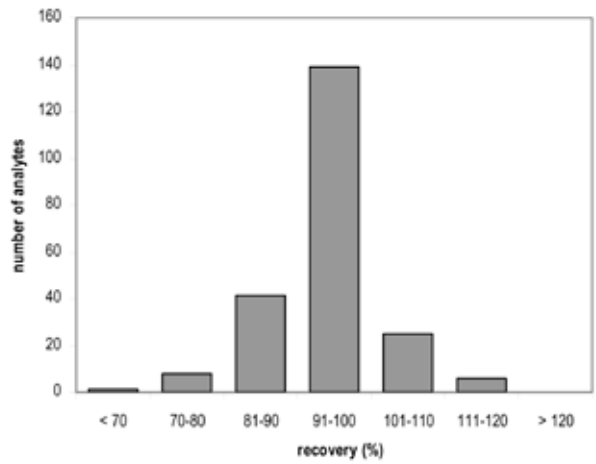

Fig. 1. **Distribution of recoveries of all 222 pesticide residues and mycotoxins at concentration level 100 $\mu g\,kg^{-1}$ in wheat**

Conclusions

The multi-toxin LC-MS-MS method for simultaneous analysis of 200 pesticide residues and 22 mycotoxins in cereals has been developed and fully validated. Acceptable recoveries and repeatabilities have been achieved for most of analytes. The trueness of generated data was also documented. The authors are convinced that this high throughput approach will find a wide use in many control laboratories in close future.

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P35 AEROBIC MTBE BIODEGRADATION BY *PAECILOMYCES VARIOTII*

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Introduction

Methyl *tert*-butyl ether (MTBE) has been used since the 1970s as a gasoline additive and octane booster to replace lead and other toxic additives and to improve combustion efficiency of gasoline. Because of useful properties, MTBE has become one of the organic compounds with the highest production in the world[1]. In 1999, about 3.3 million tons had produced in the European Union[2]. In the United States, in 1998, MTBE was the fourth-most-produced chemical[3]. However, its extremely water solubility, mobility and volatilization have resulted in its contamination of surface soils, groundwater and sediments mainly from leaky tanks and spills[4]. A report by the U.S. Geological Survey identified MTBE as the second most common contaminant of urban aquifers in the USA[5]. The U.S. Environmental Protection Agency[6] has listed MTBE as a possible human carcinogen and recommended concentration in drinking water below 40 $\mu g\,dm^{-3}$.They also proposed the methods for removal of MTBE from environments.

Bioremediation is methods, which use natural biological activity of microorganisms and plants to transform or destroy different toxic contaminants[7]. Previous bioremediation studies reported little or no biodegradation of MTBE under aerobic[8,9] and anaerobic[10,11] condition. However, authors[4,9,12,13] reported that pure and mixed bacterial cultures have been capable for MTBE biodegradation. There are only a few reports of fungal strains capable of biodegradation of MTBE[14,15,16] so far.

The aim of this paper was to investigate the capacity of fungal strain *Paecilomyces variotii,* isolate 129b, for MTBE biodegradation at different MTBE concentration in laboratory conditions.

Material and Methods

Isolation and Identification of Fungus *Paecilomyces Variotii*

The fungal strain of *Paecilomyces variotii*, isolate 129b, was isolated from the wastewater of API separator in Oil Refinery Pancevo, Serbia. The cultures maintained onto rose bengal streptomycin agar (RBSA) plates[17] at 26 °C. The identification based on fungal morphological characteristics growing in malt agar and Czapek yeast agar (CYA), using the key for identification[18].

Degradation Experiment

Liquid suspension cultures grown axenically in 250ml glass bottles. The growth medium (80 ml) was mineral salts medium[14]. The medium sterilized at 121 °C for 15 min and inoculated with 10 % (v/v) of suspension of *Paecilomyces variotii* conidia (13.8×10^5). After addition of different MTBE concentrations, the liquid suspension cultures incubated for 19 days at 26 °C and shaken at 120 rpm. All the experiments were conducted in triplicate.

The Yield of *Paecilomyces Variotii* Mycelia

The yields of mycelia measured after 19 days by means of dry weight. Liquid suspension cultures were vacuum filtered onto filter papers. Mycelia and filters were dried at 65 °C for 24 h and reweighed. The control variant was without MTBE as an sole energy and carbon source. The experiments were conducted in triplicate.

Analytical Methods

The MTBE used in these experiments originates from industrial facilities of Oil Refinery Pancevo. Consumption of MTBE was monitored by a Agilent Technologies 6890N gas chromatograph (GC) fitted with a flame ionization detector. A 30 m × 0.53 mm ID, 3.0 μm DB-624 column was used (J&W Scientific, Folsom, CA, USA). The temperature program was 50 °C for 2 minutes, ramp 8 °C min^{-1} to 100 °C, hold 3 minutes. The injector temperature was 170 °C and detector (FID) temperature 300 °C. The flow hydrogen was 40 ml min^{-1}, flow air 450 ml min^{-1} and make up gas N_2 25 ml min^{-1}.

The headspace (Agilent 7694E Head Sampler) was: vial equilibration time 30 min; bath temp. 80 °C; valve/temp loop 85 °C; transfer line temp. 120 °C; loop size 1 ml; pressure time 0.00 min; loop fille time 0.050 min; loop eq. time 0.05 min; inject time 1.0 min. Internal standard was acetonitrile (retention time 2.951 min). The MTBE retention time was 3.310 min. In experiment, headspace samples (100 μl) were taken with gas-tight syringes.

The consumption of MTBE was measured in the beginning of the experiment and after 5; 12; 15 and 19 days.

Results

The soils of Oil Refinery Pancevo are heavy polluted with different organic substances and it can be expected to find out big diversity of fungus and bacteria whose use pollutants there as sole source of carbon and energy. During different experiments, about 40 bacteria and 14 different fungi strains were identified and isolated. One of the fungi founded there, after testing, showed high ability to consume MTBE. That fungus is isolated and identified as *Paecilomyces variotii*, isolate 129b, and used in experiments. Ours results showed that degradation rate was depending on initial MTBE concentration (10.85; 34.34 and 83.15 ppm used) and time

of sampling. After 19 days of incubation, the degradation of initial middle (34.34) and high (83.15) MTBE concentration stopped, while the degradation of lowest MTBE concentration (10.85) continued after incubation period (Table I).

Table I
The decreasing of MTBE concentration (ppm) caused by fungal strain *Paecilomyces variotii* isolate 129b

MTBE [ppm]	5th day	12th day	15th day	19th day
10.85	6.6	6.08	2.45	1.55
34.34	33.5	23.34	19.19	20.2
83.15	74.3	46.92	38.25	40.7

Moreover, even different pattern of degradation the fungal strain of *Paecilomyces variotii*, isolate 129b, decreased MTBE concentration in all treatments used (Table II). The highest degradation rate (2.23 ppm per day) was in the treatment with the highest concentration used, (from 83.15 to 40.70). In the middle treatment, the degradation rate decreased to 0.744 ppm per day and in lowest concentration the degradation rate further decrees to 0.490 ppm per day. However, the total MTBE degraded by fungi is to be in highest concentration used (42.7 ppm for 19 days). From the Table III it can be seen that there is no substantial change in the fungus yields (mg/ml) compared treatments and control.

Table II
The MTBE degradation rate [%] caused by fungal strain *Paecilomyces variotii* isolate 129b

MTBE [ppm]	5th day [%]	12th day [%]	15th day [%]	19th day [%]
10.85	39.2	44.0	77.4	85.7
34.34	2.4	32.0	44.1	41.2
83.15	10.6	43.6	54.0	51.1

The highest yield of *Paecilomyces variotii* mycelia was noticed at lowest initial MTBE concentration. The addition of MTBE slightly affects mycelia yield and is similar to control. The results of mycelia yield is in correlation with degradation results, because the highest mycelia yield is noticed in variant with lowest concentration used, but even that the difference among treatments are not statistically significant.

Table III
Paecilomyces variotii mycelia biomass after 19 days of MTBE treatments

MTBE initial concentration [ppm]	repeats [mg ml^{-1}]			average [mg ml^{-1}]
	I	II	III	
10.85	0.010	0.026	0.037	0.024
34.34	0.019	0.022	0.012	0.018
83.15	0.018	0.025	0.014	0.019
control	0.013	0.022	0.016	0.017

The results of experiments showed that the fungal strain *Paecilomyces variotii* isolate 129b, is capable of MTBE utilization as sole source of carbon and energy to support growth. The highest MTBE degradation rate was at the lowest initial MTBE concentration. The addition of MTBE had a negative effect on degradation rate but total MTBE degraded was higher.

Discussion

Fungi are highly successful in survival because of their great physiological versatility. Their ability to produce extracellular enzymes is of great survival value. Fungi are involved in the biodegradation processes of undesirable materials (waste, pesticides, detergents, oil spills etc.) into harmless products[19]. The utilization of filamentous fungi for bioremediation processes has been limited compared with bacteria. Because of enzyme production in different environmental conditions, the fungi perform the degradation of broad spectrum of pollutants[20]. One of enzyme that is responsible for MTBE degradation is alcohol dehydrogenase[14], who affects the TBF formation during the MTBE oxidation. Because of its gelatinolytic and cellulolytic activity, the fungus *Paecilomyces variotii* is capable of biodegradation of some aromatic volatile organic compounds (VOC) like toluene[21], formaldehyde[22] and alkylbenzenes[23]. In addition, recent reports showed that *Paecilomyces variotii* could be use in cadmium biosorption[24] and for bioremediation of aflatoxine[25]. Previous mycoremediation studies indicated that different fungal cultures have capability of MTBE biodegradation[15,16].

The results of investigation shown that *Paecilomyces variotii* mycelia can grow in the presence of different MTBE concentration. MTBE degradation rate is lowest in the latest phase of incubation period. This conclusion is in accordance with previous MTBE studies[14], where the MTBE degradation rate progressively declined during the mycelia incubation with MTBE alone. Our experiments showed that the degradation rate was lower than in previous studies[14], but the initial MTBE concentration was lower than in our investigation. The slower degradation rate was also possibly due to presence of methanol, which was solvent for MTBE. Other authors[15] also reported similar conclusion.

The low degradation rate and yield of mycelia indicates that MTBE may be a poor substrate and energy source and/or that an intermediate during degradation may inhibit the microbial growth[12]. During the MTBE degradation, intermediates concentrations increased, while concentration of MTBE was decreased[26]. This conclusion confirms the MTBE degradation by microbial pure cultures. The results showed that ether bonds could be cleavage by certain microorganisms, although some investigations showed that this bond is not biodegradable or resistant to biodegradation[27]. According to this conclusion is a result of yield of *Paecilomyces variotii* mycelia, which was comparatively low. The low yield of microbial cultures in the presence of MTBE was also reported in earlier studies[28], probably because this compound is metabolic and electron transport inhibitor[9].

This investigation confirms that the fungus *Paecilomyces variotii* isolate 129b can utilize the MTBE as a sole source of energy and carbon and could be use in degradation of recalcitrant contaminants such as the fuel oxygenates.

Conclusions

Based on these results following conclusions are obtained:

- The degradation capability of *Paecilomyces variotii* isolate 129b depend on initial MTBE concentration and on time of sampling.
- During the experiment, the highest degradation rate was conducted at lowest initial MTBE concentration. After incubation period, the degradation of initial middle and high MTBE concentration stopped, while the degradation of lowest MTBE concentration continued.
- The highest yield of *Paecilomyces variotii* mycelia was notices at lowest initial MTBE concentration, but even that the difference among treatments are not statistically significant.
- The fungus *Paecilomyces variotii* isolate 129b can utilize the MTBE as a sole energy and carbon source and could be use in degradation of recalcitrant contaminants such as the fuel oxygenates.

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P36 BIOLEACHING OF ANTIMONY MINERALS BY BACTERIA *ACIDITHIOBACILLUS FERROOXIDANS* AND *DESULFOVIBRIO DESULFURICANS*

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Introduction

In several gold ores, gold is trapped in the matrix in of metallic sulphides (FeS_2, Sb_2S_3 etc.). In such cases recovery of gold from refractory ores requires a pre-treatment to liberate the gold particles from the host mineral. For the pre-treatment of gold ores exist several hydro- and biohydrometallurgical processes[1]. The fundamental of biohydrometallurgical processing for sulphide minerals is the application of microorganisms, which on the basis of their metabolic processes can increase or decrease mobility of metals[2,3,4].

In the area of biohydrometallurgical processing of gold-bearing antimony sulphide minerals and concentrates the iron- and sulphur-oxidising bacteria have the very important function as well as on the ground the new knowledge also the sulphate-reducing bacteria[5,6,7]. Using involved bacteria to catalyse the breakdown of sulphides that host the gold is an important biological method for the pre-treatment of refractory gold ores. Following this biological treatment a combination of chemical and physical methods are used for leaching (e.g. the cyanide process) and concentration (e.g. the electrowinning) of gold. Although these methods are well accepted by industry, they harbour limitations in the processing of low-grade refractory ores, and regulatory agency/public acceptance of cyanide use. The objectives of this work were to evaluate the use of iron- and sulphur-oxidising bacteria *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) and sulphate-reducing bacteria *Desulfovibrio desulfuricans* (*Dsv. desulfuricans*) in the biohydrometallurgical processing of gold-bearing antimony sulphide minerals. Experiments were conducted at laboratory scale on a refractory gold-bearing stibnite coming from Santa Rosa de Capacirca Mine, Bolivia. Involved bacteria were used separately at different conditions for the pre-treatment of the aforementioned sample in order to increase the subsequent gold recovery during cyanidation processes. The *At. ferrooxidans* application is based on the their ability to oxidize and dissolve pyrite and stibnite, thus releasing the entrapped gold particles. The using of the bacteria *Dsv. desulfuricans* is based on their ability of the bacterially H_2S production for the alkaline leaching of stibnite.

Experimental

Microorganisms

In the experiment were used bacteria *At. ferrooxidans* isolated from the acid mine water[8] (deposit Smolník, Slovakia) and *Dsv. desulfuricans* isolated from the potable mineral water[9] (Gajdovka spring, Kosice, Slovakia). The isolation was performed by the modified dilution method[10].

Samples of Ore

The sample of gold-bearing antimony sulfide minerals used was obtained from Bolivia[6] (Santa Rosa de Capacirca Mine). Mineralogical characterisation by X-ray Diffraction (XRD) showed the presence of quartz (SiO_2), stibnite (Sb_2S_3) and pyrite (FeS_2). Their chemical composition includes 21.93 % Si, 4.94 % Sb, 4.28 % Fe and 3.77 % (S). Quantitative chemical analysis was performed by Inductively Coupled Plasma Spectrometry (ICP).

Bioleaching Test by Bacteria

Bacteria *At. ferrooxidans* and *Dsv. desulfuricans* were used separately. The experimental tests were conducted in a fed batch reactor at 30 °C under aerobic and dynamic conditions (with *At. ferrooxidans*) and anaerobic and static conditions (with *Dsv. desulfuricans*). The weight of the sample of gold-bearing antimony sulfide minerals was of 10 g. The total volume of feed solution consisted of 100 ml selective nutrient medium[1] 9K(A) for *At.f.* with pH 2.5; and DSM-63 medium[10] for *Dsv. desulfuricans* with pH 7.5. The abiotic control was carried out without the bacteria application at the same conditions. Dissolved metals in liquid phase during the experiments were determined using an atomic absorption spectrophotometer. After 120 days the solid phases were recovered by filtration and were saved for the consequently test of cyanidation.

Results

The results of the bioleaching i.e. the pre-treatment of gold-bearing antimony sulphide minerals are demonstrated by Figs. 1.–4.

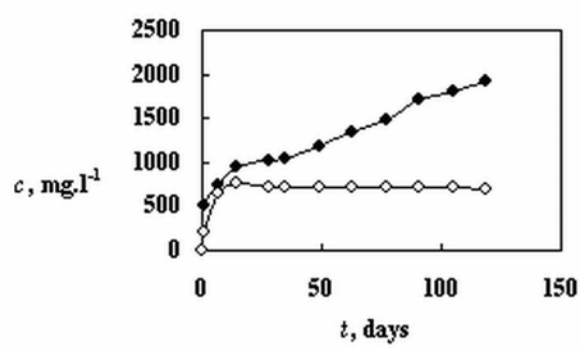

Fig. 1. **Dissolution of Fe by bacteria *Acidithiobacillus ferrooxidans* from the sample of gold-bearing antimony sulfide minerals. c – concentration of Fe, t – time of bioleaching, ● – Acidithiobacillus ferrooxidans, ○ – abiotic control**

Fig. 1. presents the dissolution of the Fe by bacteria *At. ferrooxidans* and in the cases from the start until the end of experiments the concentration of Fe in liquid phase increase. From beginning, probably due to interaction between

the mineral and the cultural medium and after 14 days by direct and indirect influence of bacteria *At. ferrooxidans.* This fact demonstrates the comparison of the Fe dissolution curve shape of *At. ferrooxidans* and abiotic control. It confirms the bioleaching of pyrite (FeS_2).

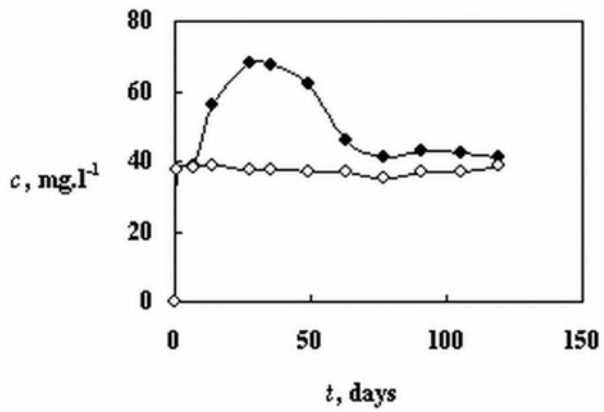

Fig. 2. **Dissolution of the Sb by bacteria *Acidithiobacillus ferrooxidans* from the sample of gold-bearing antimony sulfide minerals. . c – concentration of Sb, t – time of bioleaching, ● – Acidithiobacillus ferrooxidans, ○ – abiotic control**

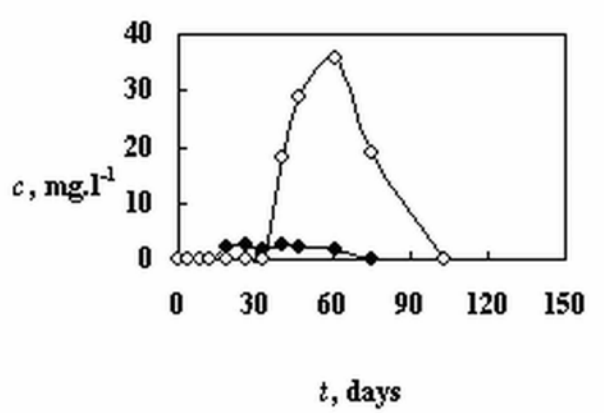

Fig. 3. **Influence of bacteria *Desulfovibrio desulfuricans* for the Fe dissolution under the alkaline leaching of gold-bearing antimony sulfide minerals. c – concentration of Fe, t – time of bioleaching, ● – Desulfovibrio desulfuricans, ○ – abiotic control**

Fig. 2. shows the trend of the Sb dissolving. Initially, during 7 days the concentration of Sb increases in the *At. ferrooxidans* presence and abiotic control, probably due to interaction between the mineral and the cultural medium. After 7 days the abiotic control allocated the circa constant Sb concentration in liquid phase until the end. After 35 days was recorded the depression as follows was observed the decreasing of the Sb concentration until the end of experiments in the *At. ferrooxidans* presence. This fact can be interpreted on the bases the creation of the insoluble antimony oxosulphates – $(SbO)_2SO_4$ according the reaction (1)[11]:

$$Sb_2S_3 + 6O_2 + 2H_2O \rightarrow (SbO)_2SO_4 + 2H_2SO_4 \qquad (1)$$

Figure 3 presents the Fe concentration in liquid phase at the using bacteria *Dsv. desulfuricans* that was from beginning until the end almost zero. Although the little increase was observed after 19 days. This situation can be resolve by the Fe precipitation with bacterially produced hydrogen sulphide under the influence of bacteria *Dsv. desulfuricans* After 40 days it's the intense increase was recorded in the experiments without bacteria. Just now concerning this fact we have not the adequate understanding. At the end of the experiments the Fe concentration at presence SRB and abiotic control were again almost zero.

Figure 4 shows the Sb concentration in the liquid phase at the using bacteria *Dsv. desulfuricans* From the confrontation of the Sb concentration curve shape in the abiotic control and in the presence bacteria you can see that the influence of bacteria *Dsv. desulfuricans* was not significant. The contamination of the abiotic control by bacteria *Dsv. desulfuricans* was not evidence. After 20 days was observed the formation of the shined cover on the reactor wall in the presence bacteria. Its can be explain to the Sb concentration decreasing.

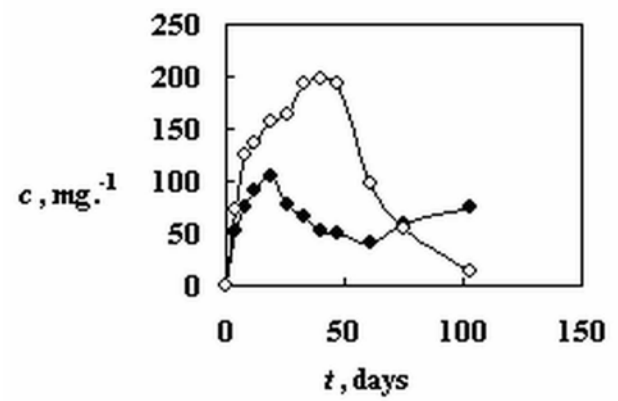

Fig. 4. **Influence of bacteria *Desulfovibrio desulfuricans* for the Sb dissolution under the alkaline leaching of gold-bearing antimony sulfide minerals. c – concentration of Sb, t – time of bioleaching, ● – Desulfovibrio desulfuricans, ○ – abiotic control**

After ending the solid phases were recovered by filtration from solution. The solid phases were saved for the consequently test of cyanidation and liquid phases for the electrowinning.

Conclusions

Experimental studies confirmed:

- the bacteria *At. ferrooxidans* have the positive influence on the dissolving of pyrite (FeS_2) and stibnite (Sb_2S_3) to occur in the used sample of gold-bearing antimony sulphide minerals,
- the increase, subsequent the depression and the decreasing of the Sb concentration during the bioleaching

experiments with bacteria *At. ferrooxidans* can be interpret on the bases the creation of the insoluble antimony oxosulphates – $(SbO)_2SO_4$,
- the bacteria *Dsv. desulfuricans* does not show the expressive influence for the alkaline leaching antimony-bearing materials.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-51-027705, Slovak Grant Agency VEGA No. 2/0075/08 and Slovak – Italian bilateral project.

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P37 THE RECLAMATION OF CALCIUM SULPHATE SLUDGES BY SULPHATE-REDUCING BACTERIA

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Introduction

Combustion of fossil fuels containing sulfur releases sulfur oxides to the atmosphere. If lime or limestone scrubbing desulfurizes combustion gases, calcium sulfates sludges are generated and these must be disposed of. Many processes for their treatment have been developed. Under appropriate conditions these sulfate can be converted to sulfide by the anaerobic bacterial sulfate reduction, which is the basic metabolic process of sulfate-reducing bacteria (SRB).

The SRB represent a group of chemoorganotrophic and strictly anaerobic bacteria that may be divided into four groups based on rRNA sequence analysis[1]: Gram-negative mesophilic SRB, Gram-positive spore forming SRB, thermophilic bacterial SRB and thermophilic archaeal SRB. They include genera like *Desulfovibrio, Desulfomicrobium, Desulfobacter, Desulfosarcina, Desulfotomacullum, Thermodesulfobacterium, Archaeoglobus*, etc.

Considering the inorganic or organic character of energy source of SRB there are two types of anaerobic respiration of sulfates authotrophic and heterotrophic[2]. SRB produce a considerable amount of gaseous hydrogen sulfide, which reacts easily in the aqueous solution with heavy metal, forming metal sulfides that have low solubility. In the bacterial anaerobic reduction of sulfates the organic substrate (lactate, malate, etc.) or gaseous hydrogen is the electron donor and sulfates is the electron acceptor.

The industrial technologies for the desulfurization of combustion products produced during the generation of electric energy by combustion of fossil fuels use limestone ($CaCO_3$) as an absorption agent. The desulfurization of combustion products proceeds in the absorber in several stages. This process results in the formation of gypsum suspension ($CaSO_4 \cdot 2H_2O$) which is incorporated into the final stored product – "stabilizate" – after being treated together with other wastes (ash, burnt lime, desulphurization waste water, etc.).

The objective of our study was to verify experimentally the possibility of using gypsum contained in the above-mentioned "stabilizate" as the source of electron acceptors for the growth of SRB with the prospect of the recycling of desulfurization agent – limestone.

Experimental

Microorganisms

A culture of SRB (genera *Desulfovibrio* and *Desulfotomaculum*) was obtained from drinking mineral water Gajdovka (locality Kosice-north, Slovak Republic). For the isolation and cultivation of these bacteria a selective nutrient medium (DSM-63 – Postgate′s C medium) was used[3].

Liquid Phase

The feed solution (the selective nutrient medium (DSM-63 – Postgate's C medium without sulfates) was prepared by dissolving analytical grade salts such as: K_2HPO_4 0.5 g dm^{-3}, NH_4Cl 1 g dm^{-3}, $CaCl_2 \cdot 6H_2O$ 0.1 g dm^{-3}, $MgCl_2 \cdot 6H_2O$ 0.3 g dm^{-3}, $C_3H_5O_3Na$ 2.0 g dm^{-3}, $C_2H_3O_2SNa$ 0.1 g dm^{-3} and $C_6H_8O_6$ 0.1 g dm^{-3} in distilled water.

Solid Phase

The sample of "stabilizate" from Vojany power plant (Slovak Republic) was used in the experiments. Mineralogical characterisation by X-ray Diffraction (XRD) showed the presence of $CaSO_4$ 40.84 %, SiO_2 22.70 %, Al_2O_3 10.70 %, Fe_2O_3 4.26 % and CaO 3.00 %.

Analytical Procedures

A turbidimetric method was used to measure the concentration of soluble sulfate ion concentrations in the liquid phase[4]. Samples were centrifuged for 10 minutes at 10,000 rpm before performing the analysis. Digital pH-meter GPRT 144 AGL was used. Qualitative changes of "stabilizate" were performed by the qualitative X-ray diffraction analysis using Dron-2 instrument and energy dispersive spectrometry (EDS) analysis using instruments, which consisted of a scanning electron microscope BS 300 and an X-ray microanalyser EDAX 9100/60. Samples of solid phase were dried and coated with gold before the EDS analysis.

Biological Utilization of Gypsum from "Stabilizate"

Series of anaerobic tests were studied in a fed batch reactor in the thermostat at 30 °C. Samples of "stabilizate" were kept in static conditions for a period of 40 days at pH 7.5. The weight of "stabilizate" was 20 g. The stock culture of SRB was used as an inoculum (10 %, v/v). The total volume of feed solution consisted of 200 ml distilled water and 300 ml selective nutrient medium for SRB (DSM-63 – Postgate's C medium without sulfates). The abiotic control was carried out without the SRB application at the same conditions. After 40 days the solid phase was filtered, dried and analyzed using the qualitative X-ray analysis and energy dispersive spectrometry (EDS) analysis.

Results

The formation of black precipitates and the sensorial detection of classical strong H_2S smell were observed after 3–4 days from the beginning of the process. These remarks were not detected in the abiotic control until the end of the experiment. Changes of sulphates concentration during the discontinuous cultivation of sulphate-reducing bacteria using gypsum contained in the "stabilizate" as the source of electron acceptors for the growth of SRB are shown in Fig. 1. The results of qualitative X-ray diffraction analysis of original "stabilizate",

bacterially treated "stabilizate" and "stabilizate" of abiotic control are shown in Figs. 2.–4. They show the significant qualitative changes in the "stabilizate" initiated by SRB. The sulfates from the original major component of "stabilizate" – $CaSO_4 \cdot 2H_2O$ (Fig. 2.) – were reduced according by SRB forming hydrogen sulfide as proved by the sensorial detection of classical strong H_2S smell. This is indirectly confirmed by Fig. 3, which proves the extinction of $CaSO_4$ and formation of $CaCO_3$ according to equation (1):

$$2C_3H_5O_3Na + CaSO_4 \xrightarrow{SRB} 2CH_3COONa + CO_2 + CaCO_3 + H_2S + H_2O \quad (1)$$

The above-mentioned changes have not been observed in abiotic control as documented by the comparison of qualitative X-ray analysis of "stabilizate" before applying SRB (Fig. 2.) with the qualitative X-ray analysis of "stabilizate" in abiotic control (Fig. 4.). The results of energy dispersive spectrometry (EDS) analysis and scanning electron microscope of original "stabilizate", bacterially treated "stabilizate" and "stabilizate" of abiotic control are shown in Figs. 5.–7. They fall into line with Figs. 2., 3. and 4. and suggest on the elimination of $CaSO_4 \cdot 2H_2O$ form "stabilizate". The element composition of solid phases (Figs. 5., 6. and 7.) accepts with this fact.

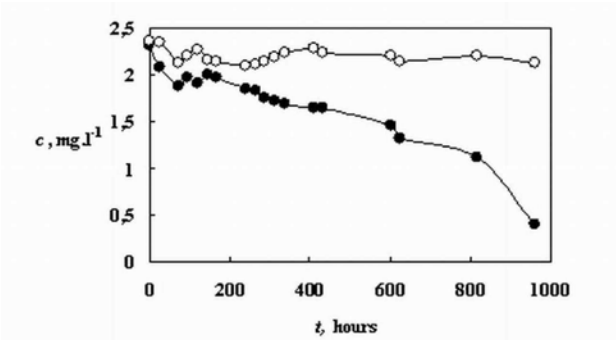

Fig. 1. **Changes of sulfates concentration during the discontinuous cultivation of sulfate-reducing bacteria.**
c – sulfates concentration, t – time of sulfate-reducing bacteria cultivation, ● – "stabilizate" with SRB, ○ – "stabilizate" without SRB (abiotic control)

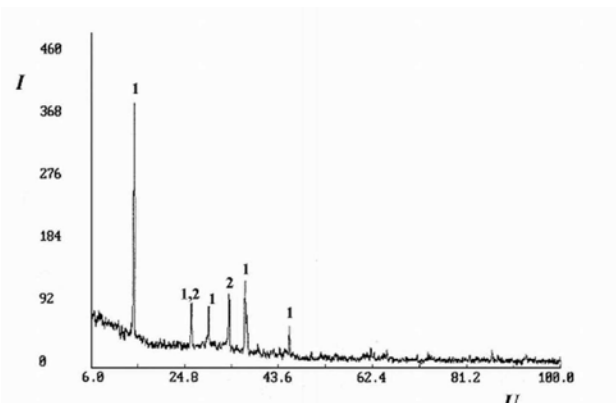

Fig. 2. **Qualitative X-ray analysis of "stabilizate" before application of sulfate-reducing bacteria. I – intenzity, U – 2-theta uhol, 1 – $CaSO_4 \cdot H_2O$, 2 – SiO_2**

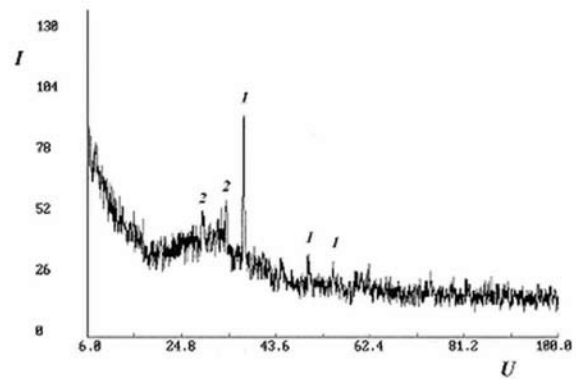

Fig. 3. **Qualitative X-ray analysis of solid phase formed by the effect of SRB on "stabilizate". I – intenzity, U – 2-theta uhol, 1 – $CaCO_3$, 2 – SiO_2**

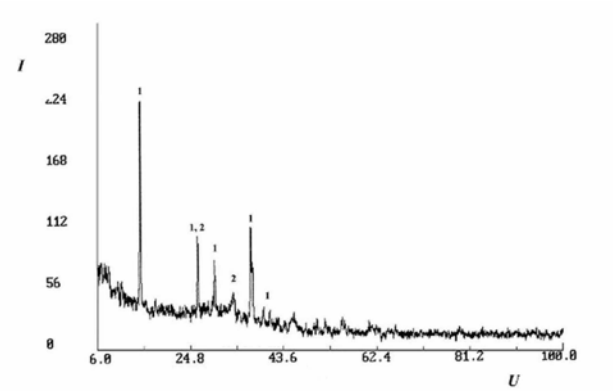

Fig. 4. **Qualitative X-ray analysis of solid phase in abiotic con-**

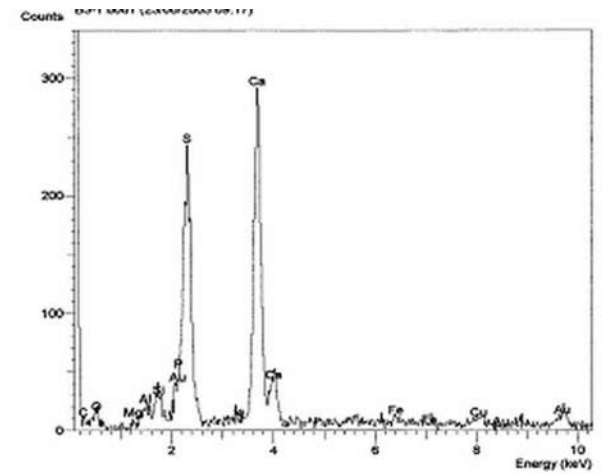

Fig. 5. **EDS qualitative analysis of "stabilizate" before application of sulfate-reducing bacteria**

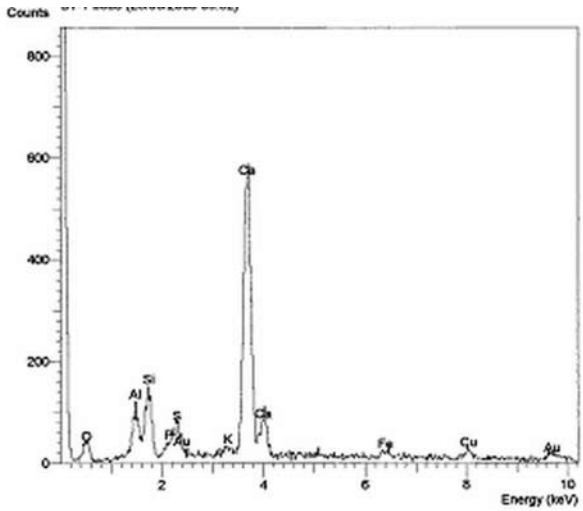

Fig. 6. **EDS qualitative analysis of solid phase formed by the effect of SRB on"stabilizate"**

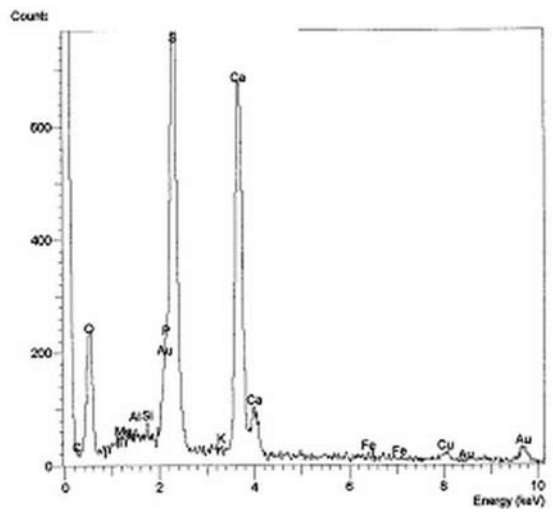

Fig. 7. **EDS qualitative of solid phase in abiotic control**

Conclusions

The results confirmed the theoretical assumptions on the use of gypsum, which forms the substantial component of "stabilizate", as the source of sulphate for sulphate-reducing bacteria, which produce hydrogen sulphide in the process of bacterial reduction of sulphates. They also showed the possibility of recycling desulphurization agent – limestone.

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-51-027705, Slovak Grant Agency VEGA No. 2/0075/08 and Slovak – Italian bilateral project.

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P38 BIOLOGICAL-CHEMICAL REGENERATION OF DESULPHURIZATION SORBENTS BASED ON ZINC FERRITE

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Introduction

The energetic demands of people in present, as well as in the future will be supplied mainly by the energy from the fossil sources, which is accompanying with the negative impact on the environment in the form of emissions of sulphate and carbonate compounds. For desulphurization and decarbonization of these emissions more commercial technological methods have been developed, to which belongs also the utilization of solid sorbents (filters) based on the zinc ferrite. The studies of Grindly[1] and Krisham[2] helped to clear up the high-temperature process of desulphurization of waste gases after coal combustion which can be described by the chemical equation:

$$3ZnFe_2O_4 + 3\ H_2S + H_2 \rightarrow ZnS + 2FeS + 4H_2O \quad (1)$$

In dependence on the conditions of the combustion process there is according to the Lamoreaux[3] a possibility of a occurring of another reaction of the iron (occurring in the solid sorbents) with waste gases of carbon, leading to formation of carbide. This is governed by the equations:

$$3FeO + CO \rightarrow Fe_3C + 2O_2 \quad (2)$$

$$3Fe + CO \rightarrow Fe_3C + ½O_2 \quad (3)$$

The phase composition of sulphurized (amortised) sorbents shows the presence of sulphidic and carbidic structures located mainly on the surface of particles of zinc ferrite (respectively Zn Fe_2O_3 – micropellets of zinc ferrite). They became inactive and the further utilization is constrained to their regeneration. In relation with the most effective methods of manifold recycling of sorbents, several methods of their regeneration were studied.

The literature shows two methods of regeneration of sulphatizing sorbents based on zinc ferrite. The first method commercially used, is a oxidising pyrolysis of sulphides[2]. In this process are the sulphidic structures in dependence on the temperature of the oxidising rousting transformed step by step at the first stage to the sulphates and at the second stage which corresponds to the temperature of sulphates decomposition (in the temperature range from 480 to 600 °C) to the oxides, according to the equations (4) and (5):

$$2FeS + 3.5O_2 \rightarrow Fe_2O_3 + 2SO_2 \quad (4)$$

$$ZnS + 1.5O_2 \rightarrow ZnO + SO_2 \quad (5)$$

The second method of the regeneration of desulphurization sorbents which is introduced by Sakao[4], is based on the principle of pressure leaching of ZnS in water environment at the temperature over 550 °C. This method is described by the equation, almost identical with the Eq. (5):

$$ZnS + 1.5O_2 \rightarrow ZnO + SO_2 \quad (6)$$

The commercially available technologies of sulphides processing and treatment (as sources of colour and rare metals) have nowadays started with utilization of biological-chemical processes (bio-leaching); where the physical and chemical base of degradation of sulphidic structures is identical with the methods of regeneration of desulphurization sorbents mentioned above.

The principle of all mentioned methods is the process of sulphides oxidation with utilization of various methods of catalysis. In the process of thermic regeneration is the oxidising reaction catalysed by the thermic energy, at pressure leaching by the pressure and temperature.

The aim of this paper is to testify the biological-chemical method as a new method of sorbents regeneration, utilising the catalytic effect of the metabolism of an acidophilous bacteria – *Thiobacillus ferrooxidans,* oxidising the sulphate and iron[8].

Experimental

M a t e r i a l

Zinc ferrite

For biological-chemical regeneration, zinc ferrite after sulphurization test was used.The powder sample containing 90 % zinc ferrite and 10 % bentonite binder by weight was pelletized to cylindrical pellets, calcined in air from room temperature to 970 K, and crushed and sieved to the size of a stainless steel cylinder, in which 25 g of pelletized sorbent was packed.The simulated coal gas had the following composition: 30 % vol. CO, 50 % vol. N_2, 19.5 % vol. H_2, and 0.5 % vol. H_2S. Total gas flow-rate diring absoption was 5,000 $cm^3\,min^{-1}$. The desulphurization tests were stopped when the H_2S concentration of the effluent gas was above 50 ppm. The details of the sulphurization experiments are described also in our previous papers[6,7].

Bacteria

The bacteria used in experiments was: *Thiobacillus ferrooxidans*, isolated from the mining drainage waters of sulphides deposits, which is cultivated for a long time on the sphalerite (ZnS) substrates. The leaching solution was formed by cultivated medium according to the Silverman and Lundgren[5], in which the cells of bacteria were scattered. The pH value of the solution at the beginning of the reaction was 1.6.

M e t h o d s

The experiments of biological-chemical way of regeneration were realized by the form of charge leaching tests at the suspension density 5 % at the temperature of 30 °C, under continuos stirring for the period of 19 days. The qualitative changes of investigated zinc ferrite during the process of biologico-chemical way of regeneration were evaluated by:

- RTG diffraction analysis (diffractometer DRON 2,0 Technsabexport, Russian, FeKa radiation),
- particle size distribution (granulometer Helos and Rodos, Sympatec GmbH Claustahl Zellerfeld),
- BET adsorption method for measuring the adsorption surface (Micromeritics, Gemini 2360, in nitrogen atmosphere),
- chemical analysis of the content of Zn and Fe in the leach (atom absorption spectroscopy on the instrument Spectra AA-30 ,Varian, Australia).

Results

RTG diffraction analysis of amortised sorbents revealed except of the rest ferrite zinc (Franklinite, $ZnFe_2O_4$), which represents probably the nonreacted cores of micropelets, also the presence of the formed sulphidic, sulphate, oxidic and carbidic compounds: b-sphalerite (ZnS), wurtzite (ZnS with addition of Fe), pyrite (FeS_2), melanterite ($FeSO_4 \cdot 9H_2O$), elemental sulphur (S), magnetite (Fe_3O_4), hematite (a-Fe_2O_3) and cohenite (Fe_3C).

The bio-leaching of this product leads to the biologico-chemical degradation of amortised layers located on the surface, which is accompanied by the extraction of Zn and Fe to the leach. The changes of the concentration of introduced elements in the leach in dependence on the time of leaching are shown in Table I.

The concentration of Zn in the leach during the observed time interval showed the arising trend, while in the Fe concentration dependence has been appeared the maximum (after 15 days the concentration of the leached ferrite decreased). As the realized experiments represent the spontaneous non-regulated process of biologico-chemical oxidation, it is possible to assume that the decrease of the Fe concentration

Table I
The time dependence of changes of Zn and Fe concentration in the leach during the biological-chemical regeneration of amortised sorbents based on zinc ferrite

Time leaching [days]	Concentration Fe [g dm^{-3}]	Concentration Zn [g dm^{-3}]
0	0.37	0.62
3	0.37	1.21
5	1.48	1.56
7	1.50	1.73
10	1.39	2.68
14	0.52	2.81
17	0.55	2.97
19	0.61	3.19

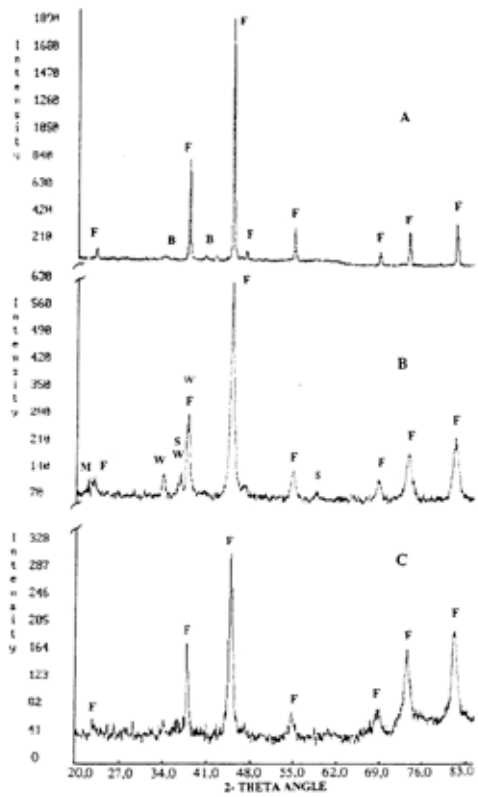

Fig. 1. **Comparison of X-ray phase analysis of zinc ferrite sorbent, A – primary, B – pasivated, C – biologically and chemicaly regenerised**
Legend: W – wurtzite, F – franclinite, S – sphalerite

was caused by the precipitation of the secondary oxididic compositions of the iron. This assumption has been confirmed by the RTG diffraction analysis of the leach. After finishing the biological-chemical leaching of amortised sorbent the extinction of the most of secondary structures formed by sorption of sulphate compositions was observed by the RTG method (Fig. 1.).

The regenerated sample contained the franklinite, addition of magnetite, metal zinc and the rest of wurtzite, which during the observed period manifested itself as refractory mineral and to its destruction the longer period or the change of electrochemical conditions of leaching should be needed[9].

Referring to the reality, that the effectivity (the sorption capacity) of sorbents is directly connected with the sizes of their surfaces and inversely with the particles sizes, the changes of these parameter were also studied (Fig. 2.). The process of biological-chemical regeneration caused intense changes in the adsorption surface of sorbents, it has changed from the value of 2.8245 m^2 g^{-1} at amortised sample to the value of 6.2543 m^2 g^{-1} at the regenerated sample.

The expressive change was also registered at the distribution of the particles sizes. The value of mean particle diameter has decreased from the value of 9 mm at amortised sorbent to 2.19 mm at the regenerated sorbents (Table II).

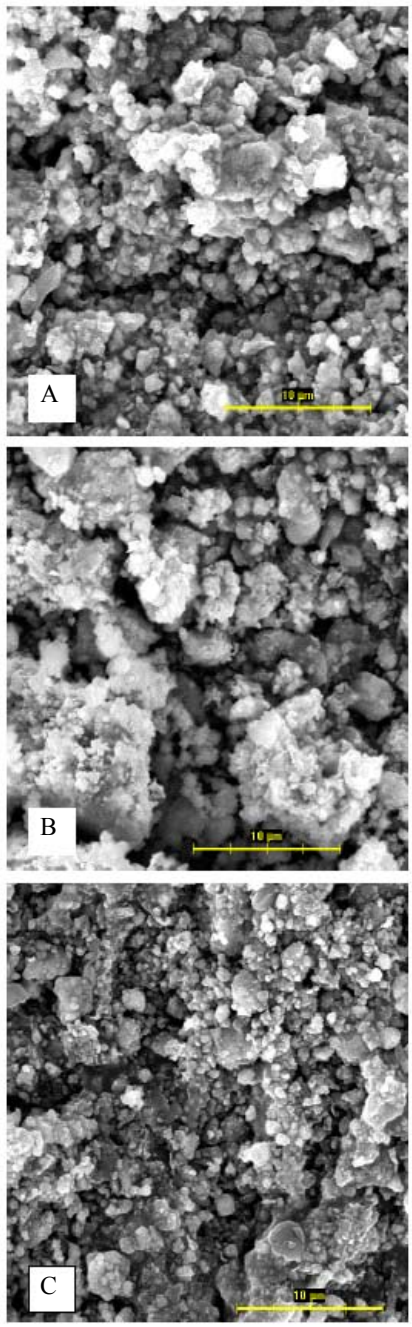

Fig. 2. **Mophology of surface of the zinc ferrite sorbent, A – primary, B – pasivated, C – biological and chemical regenerised**

Conclusions

The presented results confirm the possibility of utilization of the biological-chemical method as a new regeneration method of desulphurization sorbents based on zinc ferrite. The big advantage of this process in comparison with conventional regeneration methods is the lowering of the temperature of leaching from 500°C to 30°C. The period of the duration of the regeneration seems to be an disadvantage, its lowering requires the additional research aimed at the continual optimalization of conditions for biologico-chemical regeneration.

Table I
Comparison of the selected fysical and chemical charakteristic of the zinc ferrite sorbents before and after biological and chemical regeneration

Sample	Specific surface SA [$m^2 g^{-1}$]	Surfaces factor f* d_{50} [μm]	Average of particles
primary sorbent	2.6	1.022	4.77
pasivated sorbent	2.8	1.478	9.10
regenerised sorbent	6.2	1.797	2.19

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-51-027705.

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P42 CAUSES OF ACCIDENTS REGARDING TRANSPORT OF DANGEROUS GOODS

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Introduction

Transport of dangerous substances can be characterized by a permanent or relative contact with the population and represents in all possible stages a potential hazard. Related to human health, injury or death people during accidents also damage to property and the affected environment, the transport of dangerous goods is very serious problem. Road – accidents in hazardous materials transportation have increased 95 % in the last 30 years. This negative feature of the excessive traffic is evident due to available statistics of the accidents in the whole Europe. Besides the noticeable number of accidents in the cases related to the transport of dangerous substances the economical point of view in increasing costs is under general concern.

The most frequented transportation regarding dangerous goods is on the roads in the Europe.

Results

Accident registration has been launched in 2000 in the Czech Republic by the traffic police. Data from 2000 to 2002 are not very precise because of administration faults. Data registered since 2003 has been more precise.

As an example of main causes of accidents the accident frequency in 2003 and 2004 were used – regarding accidents conform to the directive ADR (European Agreement Concerning the International Carriage of Dangerous Goods by Road). There was a new code system regarding causes of accidents used. Previous code system included causes and their consequences related to the hazard. The new system is focused only on the main cause of accident. The aim of the system is the minimal and concise list of codes.

All results regarding causes of accidents were worked out by cooperation with Police of the Czech Republic.

Figs. 1. and 2. show the main causes of ADR accidents which are evident.

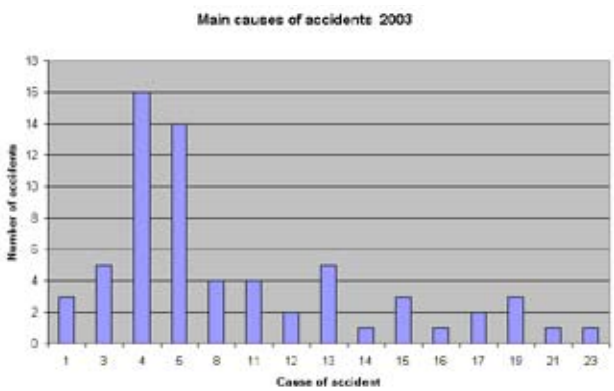

Fig. 1. **Main cause of accident, 2003**

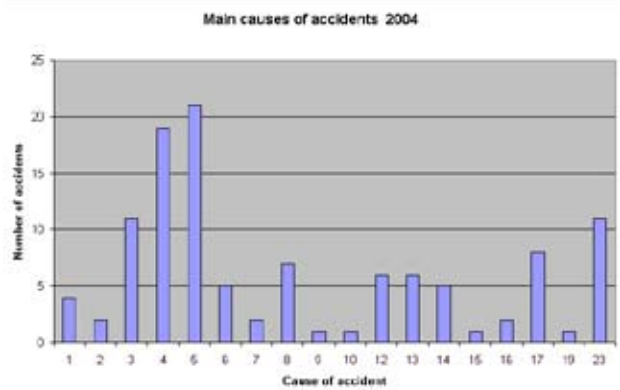

Fig. 2. **Main cause of accident, 2004**
1 – overtaking, 2 – side-on crash, 3 – speeding (not keep a safe distance), 4 – driver did not adapt speed (to condition and character of a road, cargo character, skills of a driver, character of a car), 5 – poor attention of a driver (skid, crashing into a barrier, off the road, collision with a car, a stationary car, a driving car, collision with a pedestrian, collision with a bicycle), 6 – while reversing, 7 – uncontrolled driving of truck set, 8 – driving roadside, 9 – not respecting railway signals, 10 – turning round, 11 – technical fault (on the vehicle, on the vehicle set), 12 – turning off, 13 – non-acquaintance with or bad judgment of the size of the truck, 14 – crash with upstream driving car, 15 – bad fixation of a cargo, 16 – falling asleep, micro-sleep, 17 – not respecting traffic signs ("right of way", prohibitive), 18 – not giving right of way, 19 – bad driving in the traffic lane (side-on crash, inadvertence of the parallel driving car), 20 – character of the road, damage of the road, 21 – contrary climatic conditions, 22 – impaired driving, 23 – cause is unknown

Reasons of accidents are mostly the same when considering small cars or trucks. The main reasons are human error; poor attention of drivers and their reactions and no acceptance of the speed limit.

The experience of drivers is crucial and each company (transporting dangerous goods according to the ADR) should have a possibility to check driver's crime sheet regarding accidents and other offences.

From the figures below the age of drivers causing accidents is evident. The statistic results from years 2003 and 2004 for construction of Figs. 3. and 4. were used.

From the mentioned results is evident, that the age of drivers has no effect on the accident frequency (ADR) or the cause. In fact many of available studies show relation between frequency of accidents (regarding total number of car accidents) and age drivers.

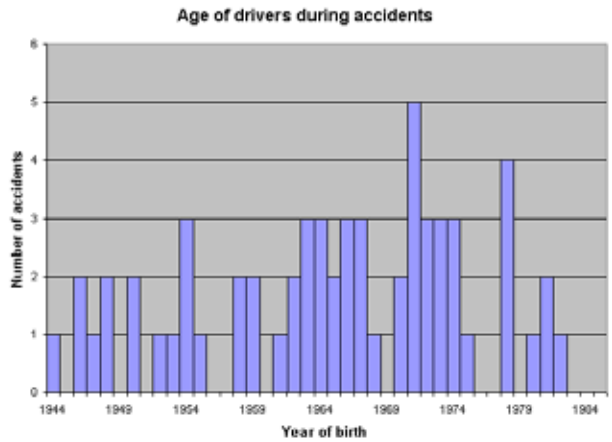

Fig. 3. **Age of drivers during accidents, 2003**

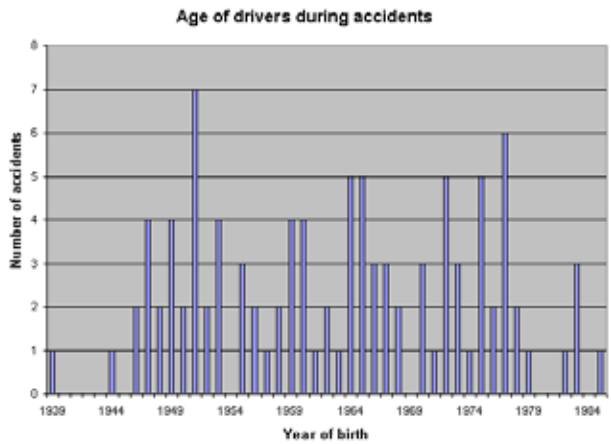

Fig. 4. **Age of drivers during accidents, 2004**

Conclusions

The Czech Republic is one of the countries with the largest accident frequency and high number of deaths of people during accidents. Reasons for accidents are mostly the same when considering small cars or trucks. The main reason is human error – insufficient attention of drivers and their reactions, further more no acceptance of the speed limit.

Substances included in the group of flammable substances (according with European ADR directive) represent the most frequently transported dangerous materials on the roads. A risk assessment for dangerous substances is performed using different kinds of methods. In order to propose and create methods for the safe transport of dangerous goods is at first necessary to perform an extensive analysis of the existing situation. Methods must be also focused, made relevant to different localities and the industry to represent different surroundings. More strict penalties could be applied. The experience of drivers is crucial and each company (transporting substances according to ADR) should have to check drivers' crime sheet regarding accidents and other offences and also prove their skills.

There is possibility to improve the situation through our preparedness, self-education, and interest regarding the problem.

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P43 RISKS FACTORS OF SOCIAL DEVELOPMENT AS ENVIRONMENT FOR MANAGEMENT

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Introduction

All things, phenomena, and processes have their own causes and consequences. They bring along pros and cons; they can be controlled and chaotic, can and cannot be influenced. Social development has been getting more and more complicated, undergoing fast changes; being difficult to predict. If the last century was said to be a century of changes, this feature will be more significant and determining for further development. There is more and more urgent need to ask questions about further development and search for answers to them. What is our future going to be like? What factors are there going to become determinants for further phenomena and processes management? It is necessary to deal with negative consequences of our decision making processes. For the purpose of this contribution, concerning the topic of the conference, We are going to try to briefly describe some of the crucial factors development, which could be considered as risks.

Authors think that everybody can be influenced by them, as well as influence them him/herself. This concerns especially those people who manage.

Risk factors

Factor 1 – Population Accumulation Dynamics

The number of the Earth population grows unevenly, especially in materially immature countries. In materially mature countries the birth rate is descending, there are a growing number of seniors. This will evoke changes in processes as well as in number and quality of human population, its behaviour and migration.

Population aging will influence its integration into economic and other socially significant processes. Social and health systems, material and non-material needs, such as boarding, dressing, hygiene, travelling, free time, home equipment; relationship between people and generations will change. Country defence potential will change, too.It is a big difference between the needs to negate poverty with a lack of capital and material production, and, on the contrary, to offer capital and material production to poverty.

Factor 2 – Inequality of the World Wealth

There are a few countries that experience a relative prosperity. On the other hand it is estimated that up to two billions of people suffer absolute poverty. There are about fifteen countries on the planet which record growth, but more than a hundred countries record descent or stagnation. According to some records there are 85 % shopping expenses for 20 % of the richest people. On the other hand 20 % of the poorest people must settle for 1.1 % of the world incomes (as compared to 1.4 % in 1991 and 2.3 in 1960).

More than a billion people do not have access to medical services, basic education or drinkable water, two billions do not have electricity and 80 % of the world population do not have the slightest possibility to telecommunicate and this access new information and communication technologies, which can, for example, enable distant studies.

It has also been found out that if the world population used the same ways of development and consumption as North America, we would need three planets like the Earth is.

The gap between the rich and the poor is getting bigger. On one side there are great chances to balance the inequalities, and on the other side they are being deepened. The problems of justice are to be seriously dealt with. The poor can not pay for the prosperity of the rich.

The inequalities are deepened not only among countries, or the North and the South, but also among people inside the countries. For instance, in the USA the rate between the highest and the lowest salaries has risen from 35 multiple up to 150 multiple within the last 20 years. We witness deepening of inequalities even here in our republic. Is it right for the poor to contribute the rich? How long will it last to the poor to start taking from the rich or at least make their wealth less pleasant? There are such cases and most probably there will be more and more of them.

Factor 3 – The Environment

Pressure caused by human activities having impact on nature systems is huge. Generally, it is connected with great material movement. Unrenewable resources are being fatigued, wasted, species of fauna and flora perish, water and air are being polluted, and number of natural as well as human-caused disasters is growing.

There have been mostly warning and non-optimistic statements published in the last few years. They are often contradictory. Yet, they have something in common – they make us think and act. Acting in the sense of adequate performance does not appear very often. It is so for many reasons.

Europe, as well as other continents, is going to face droughts and floods. Water level of seas can rise up to one meter. There is a threat of devastating winds. World map is expected to change.

According to the Living Planet Report, published by the World Wildlife Fund (WWF), standards of our lives can collapse in three decades. It is because we have been withdrawing from the planet every year up to one fifth more than the planet is able to renew. If the development continues this way, the biological capacity withdrawal deficit will reach up to 220 % in the half of the century.

The report states that the life expectancy will rapidly decline around the year 2030. Level of education and world economy will irrefutably collapse.

Unrenewable resources are being fatigued and wasted, species of fauna and flora perish, water and air are being polluted, and number of natural as well as human-caused disasters is growing. Environment knows no frontiers. In the past years at international conferences attention was paid to greenhouse gases emissions, yet no success was reached. For example – the USA produce 2.5 multiple of carbonic oxide emissions per person than Europe. In the USA the emissions production growth is eighteen per cent; the world average is nine per cent.

A hectare of tropical forests disappears every two seconds. The species disappear from hundred to thousand times faster then they would when living in a harmonic environment.

Water problems are increasingly urgent. What may happen is that this century will even become a century of a war over water, food and raw materials. Water means life. Lack of water is more and more apparent. Rivers, streams, swamps and moor land dry up.

Unrenewable resources are being fatigued, wasted, species of fauna and flora perish, water and air are being polluted, and number of natural as well as human-caused disasters is growing. Environment knows no frontiers. In the past years attention was paid to greenhouse gases emissions at international conferences, yet no success was reached. For example – the USA produce 2.5 multiple of carbonic oxide emissions per person than Europe. Water problems are increasingly urgent. Maybe this century will even become a war over water, food and raw materials.

Factor 4 – Globalization

Consequently to almost complex globalization the world is really or relatively diminishing, with no regard to continents, countries, districts. Mutual dependency of population is growing incredibly; consequences of individual's mistakes significantly affect others.

Here an essential question is to be asked: What is the connection between often absurdly defended freedom of individuals and at the same time the growing mutual dependence of people? Apart from freedom there is also justice and stability, balance and equality. So far we have been witnessing more of globalization of power, profit and exploitation. It is necessary to globalize responsibility for nature and people in it.

Great attention is being paid to globalization in various discussions. It has its positive features as well as big negatives. We must find a way to tame it to avoid a great disaster.

If we manage to run the globalization processes there is a chance for a positive development. It is good that Movements against wild globalization start to appear.

Factor 5 – Integration and Disintegration

Various groups come into being and on the contrary, states decay, therefore the there is bigger and undesirable atomization. People, regions and countries have their own interests, often of vital importance, which can be and are in contradiction with interests and aims of other countries. It is difficult do make compromise solutions. Politics and economics deal more often with the consequences than causes. Almost 20 new countries have arisen within the past 10 years, which have different political systems and aims, which are, apart from other, based on nationalistic principles.

The role of international organisations changes – UN, EU, International monetary fond, World Bank, WTO, NATO and others. We can not say that the changes are always positive, transformation is essential.

Factor 6 – NCB Weapons

There are a growing number of countries which have or will have nuclear weapons. That increases the world insecurity as well as opportunities to destroy. Here is important to note the proliferation of military equipment and technologies of ambiguous use, like NBC weapons. Gun trade volume is not low. For example, in the last three years, American companies sold weapons for nearly 19 milliard dollars; French companies for 4 milliards dollars, and German ones for 1 milliard dollars.

Besides official, by governments approved gun trades there is a trade that is being tolerated and concluded by governments. There is also illegal gun trade.

A big threat of today is a black market dealing with nuclear material and nuclear equipment. Gaining theoretical knowledge in the way of overpaying the specialists or documents is a current problem, too. Non-proliferation of all kinds of weapons, especially NBC weapons has another side, too. It is that they are kept by countries that have already had them. The logic is simple – why do some countries have them, and why the others do not? Why can you have them but we cannot? Once these weapons exist, it is a question of time and money for the others to get them. The "others" can potentially mean terrorists. Why have these weapons actually been developed and why do they exist? If the development does not change, the world will take a risk of self-destruction. The danger can be bigger than it was during the cold war time. During the cold war time, these weapons "helped" keep peace, though the peace was unstable and faulted.

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Factor 7 – Violence Growth

It is easily provable that there is a quantitative as well as qualitative growth of violence. Here it is appropriate to remember Erich Fromm who defines violence as an urgent

consequence of a non-lived and crippled life. A person who is not able to create must destroy. We have been witnessing growth of violence. The violence has many forms – human (physical and mental), military, economy, financial, political, etc. Difference between war and peace has been becoming less apparent as seen from many contemporary examples. The volume of gun-running is not small. For example, in the last three years American companies sold weapons for nearly 19 milliard dollars, French companies for 4 milliard dollars and German ones for 1 milliard dollars. People tend to feel insecure. Violence has negative influence on mental, physical, and social health.

Factor 8 – Permanent Development

Probably never in human history was people's ability to self destroy as high as it is today. Will we control the ability or cope with it? Is permanent development possible? Is there and will there be enough sources for needs filling? Values and needs vary with individuals, regions, states and continents – will the differences be apparent? One of many world principles is stability and balance. Let us admit that something like this exists in materially developed world. The situation, however, can rapidly change into misbalance and dramatic value, visions and ideology conflicts.

The world has been changing fast and dramatically. Is mankind able to accept these changes throughout its physical and mental essence? Probably everybody must admit that mere existence of modern technologies brings along civilisation risks that were unthinkable several years ago. How can the modern technologies be used and controlled for general use? Do modern technologies serve people or do people serve modern technologies?

Factor 9 – Ability to Manage

To be able to cope with development and suppress negative factors as much as possible, the problems have to be recognised and described openly and impartially. It also necessary to find consensual solution – to manage. Management is, in its essence, optimal use of available sources. The main group of sources include: human, financial, material, information and time sources.

Risk factors are unpleasant parts of life. They can, however, work as challenge for searching for solution. Individual and social scruples make people tend to avoid thinking about unpleasant things. This phenomenon can be seen every day. However, burying heads into sand and pursuing ostrich-like policy will not lead to success. This policy will lead to postponing and not solving the problems and, in its consequences, will be nonreturnably harmful.

It is necessary to independently examine the risk factors and, if necessary, to establish an independent institution for these purposes. Avoiding unpleasant things can cause their outbreak. Political institutions have to be included in this work in larger extent than they are today. Without wearing ideological blinkers must we ask questions like: "Is it true? Can it be true?" instead of "who and why said that?".

If we characterise contemporary and, especially, future environment as turbulent, it will not be an empty or just interesting concept. In various studies there have been characterised dozens of risks that can reach different levels of significance and different levels of development. It is probable that there may quickly appear new unexpectable, and, from today's point of view, unpredictable risks. Unfortunately, we make some decisions using logic of today or even yesterday. Environment has been becoming more and more complicated; technologies have been in progress. People and their understanding, thinking and behaviour are changing, too.

It is necessary to ask the main question: Is there (do we have) any visions, plans, and directions for the new century? Are there any devices for them? Don't we focus on short-term, purpose-made, selfish solutions, which will chase us into a trap? Don't we remove consequences instead of causes of our decisions?

We are certain that the crucial key matter of future is regulation, management, ability and willingness to manage even within international standards, as well as balanced unity of managing items. It is necessary to solve problems within international standards without selfishness, using the principles of solidarity and balanced values substitutions. It will take us a long time to do so.

Conclusions

The above stated factors are mutually interconnected; they influence each other, change their content and extent. There is a possibility of chaotic and unrestrained development along with uncontrollable, unpredictable changes resulting from these factors. In their consequences, these changes create risks and threats. It is very important for the top management representatives to realise the characters of development factors. These factors concern each of us, too.

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P44 CAN WE ENSURE SAFER ENVIRONMENT FOR CULTURAL HERITAGE?

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Introduction

Books should be kept in a stable environment. This is best effected by preventing temperature changes around the collection or ensuring that any changes made will be very gradual. In addition, books in fragile condition should be placed in close-fitting, nearly airtight enclosures. They protect materials from dust and airborne pollutants; reduce the exchange of moisture between the paper in the books and the air. Slow changes in air temperature around a book will not cause harm, even sudden upward temperature changes are not too damaging. In case the book in a protective enclosure is suddenly cooled, water will condense on the enclosure's interior walls when the temperature drops bellow the dew point of the air within the enclosure.

The climate maintained in a library is the result of a compromise between the needs of the readers and the staff, maintenance demands and the structure of the building which should result in minimizing the deterioration rate of the collection. It is believed that that the rate of deterioration of library materials doubles with every 10°C increase of temperature. This belief is based on the fact that the speed of chemical reactions depends in large part of temperature. Recommended temperature ranges for a variety of library materials.

A certain amount of relative humidity is necessary for paper to retain its flexibility but scientists disagree about the optimum relative humidity desirable because increased moisture content increases the rate of deteriorative chemical reactions and mold will grow. The recommended level of relative humidity is a compromise among several requirements:

- level of moisture high enough to maintain flexibility,
- level low enough to slow deterioration of materials and control insects and mold,
- level that will do no structural harm to library buildings due to condensation in cold weather.

Molds cause a downy or furry growth on the surface of organic matter; they can develop on leather, cloth, paper, etc., especially in the presence of relatively high heat and relative humidity. Every cubic meter of air contains thousands of molds spores that cover surface of library objects. Mold and mildew eat books and papers. The cellulose, adhesives and starches in the sizing provide a source of nutrition that enables the fungi to excrete digestive enzymes that convert these materials into forms they can digest. Molds usually attack bindings before the text block because it lands on the binding first, the cellulose is more difficult to digest and the text block is tightly closed.

Salvage of Flood Damage Papers

It should be noted that flood damage to some items may be irreversible. The treatment of objects of high monetary, historic or sentimental value should only be performed in consultation with a conservator.

Many people are sensitive to mold and some mold species are toxic. The best way to prevent or stop the outbreak of mold is to remove items from environmental conditions that encourage mold growth: high temperature, high relative humidity, stagnant air and darkness. If wet and moldy materials cannot be dried immediately they may be stabilized by freezing. Placing damaged items in a personal or commercial freezer will not kill mold, however, it will put the mold in a dormant state until time and the appropriate treatment environment are available. Active mold look fuzzy or slimy. Dormant mold is dry and powdery. Mold which remains active after freezing or after the host material appears dry may be treated with exposure of 1–2 hours to ultraviolet radiation from the sun. Extreme caution must be exercised when treating material outdoors: too much radiation will accelerate deterioration and may cause fading, wind may cause physical damage, and high relative humidity or condensation caused by rapid temperature changes may also exacerbate mold growth. Dormant mold spores will reactivate as soon as conditions are favorable. They should, therefore be removed from items and may be brushed or vacuumed away.

There are both chemical and non-chemical means to kill mold. Effective treatment can be fungi-static or fungicidal. Fungi-static treatments are those preventing the mold spores from germinating but do not kill the mold. Freezing is one of methods. Fungicidal treatment kills the mold and its spores. No safe large-scale treatment imparts lasting or residual mold control. That is why it is important to change the environment so it inhibits mold growth. In addition, there is some evidence that books and papers treated with fungicides may be more susceptible to mold after treatment than they were prior to the outbreak.

Fig. 1. **Flood damaged book**

Paper is very fragile when it is wet. In some cases it may be desirable to remove caked-on mud and dirt as dirt left by receding water may be contaminated. Wet documents or photographs which cannot be air dried within two days should be frozen to inhibit mold growth. Circulating air will effectively dry most items. Physical distortions may result but information will be saved. Blotting materials for air drying should be clean and absorbent. Screening material such as window screens, well supported and stacked with spaces between them provide an excellent compact drying surface. The porous surface assists air circulation and promotes drying.

Without intervention glossy materials such as paperback book covers, art books, etc. are likely to stick together. Loose glossy materials should be spread out in one layer for air drying. Bound glossy materials must be interleaved between every page to prevent sticking.

As to books, interleaving material should be placed between the text block and the front back covers. If time and supplies allow interleaving material should be placed intermittently throughout the text as well. Evaporation of water as it wicks into the interleaving paper will enhance drying.

Several classes of photographs are highly susceptible to water damage and the recovery rate will be very low. Old photographs and negatives can never be frozen. Most prints, negatives and slides may successfully be individually air dried face up. Contemporary photographic prints and negatives which are still wet and have stuck together may separate after soaking in cold water, however, this type of treatment could cause irreversible damage. Highly valued items, particularly prints, for which there is no longer a negative, should be referred to a conservator immediately.

Conclusions

The most critical element affecting the longevity of library materials is the environment in which they are used and stored. The sitting of the building, its orientation to the sun, building's location in areas safe from flooding and other natural disasters, planted areas and trees near perimeter walls, the design of roofs, basements, and location of windows considering stack areas. But it is not only buildings and their design that cause problems: libraries house millions of books published on acidic paper, high temperatures and humidity cause chemical reactions between the cellulose in paper, the acids residing in the fibers, and pollutants in the atmosphere, all of which accelerate deterioration.

Adjusting the environment near the building can help considerably in reducing problems inside.

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P47 CIVIL PROTECTION IN THE CZECH REPUBLIC AND ITS PERSPECTIVES

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Introduction

From the legal, institutional and structural points of view the history of the conception of civil protection in the Czech Republic was relatively complicated during 90-ties. The above development accompanied fundamental social-political changes in the country after 1989 without being completed with adaptation of legislation focused on civil protection. Even if the importance of civil protection reflecting increasing risks of anthropogenic, natural and mixed origin in peace conditions was fully accepted by specialists and the responsible state authorities passed individual measures aimed on gradual change of the state then, the global conception of civil protection was approved by the Czech government as late as in 2002.

The Act No. 239 of June 28, 2000 on integrated rescue system introduced the term civil protection into the Czech legal code. According to the above act civil protection is understood as fulfillment of civil protection tasks, especially warning, evacuation, sheltering and emergency survival of civilian population and further measures to ensure protection of their lives, health and possessions with reference to the article No. 61 of Protocol Additional to the Geneva Conventions of August 12, 1949 on protection of international armed conflicts victims (Protocol I).

Civil protection has become an integral and priority domain of emergency planning and management. In connection with its systematical implementation and practical realization the necessity of university education for qualified emergency managers mastering thoroughly both theoretical and practical background of civil protection has been proved.

History of Civil Defense and Protection

Purposeful and qualified attention has been paid to the practical and theoretical problems of civil protection in the Czech Republic (former Czechoslovakia) for more than seventy years.

As early as in 1929, after World War I, the Centre for Civil Defense was established by the Czechoslovak Ministry of Defense as a response to both complicated international political situation then and to growing fears of danger from air force war activities threatening civilians especially with chemical warfare agents. The Centre was run as a voluntary organization via local bodies with the semi-official support of central authorities and with active participation of organizations whose program was concentrated on defense activities. One year later the organization Protection of Civilians against Air Raids arose.

A remarkable change of situation was noticed in the first half of 30-ties of the previous century. At that time the attempt of Nazi Germany to realize its political aims aggressively by all the available means expressed by its intensive building military and air forces was becoming more and more apparent.

The authorities of the Czechoslovak Republic reflected the above situation by introduction of a series of individual precautions resulting in passing the Act No. 82/1935 Coll. on protection from and on defense against air raids. The base for system of legal regulations for civil protection was set both by promulgation of the above law and by establishment of Civil Air Defense (referred to as CAD). Amending the above law another Act No. 75 of April 1938 represented a reaction to immediate threat of the Czechoslovak Republic by fascist Germany. The conception of CAD in the pre-war Czechoslovak Republic fulfilled requirements then and under given conditions it comprised the first historical phase of systematic and effective attempt to solve the problems of civil protection.

During the occupation of the Czechoslovak Republic by Nazis the CAD was liquidated and its compartments, units and material equipment were taken over by the German Air Defense in 1941. After liberation in 1945 the CAD passed out of existence. The first attempts to re-establish it were realized after 1948.

Regardless of certain individual measures the work on institutionalization of a civil defense (referred to as CD) organization culminated in 1951 by acceptation of Government resolution on civil defense fixing gradual organizational, personal and material building of civil defense in the legal code.

Between 1951 and 1970 civil defense was focused solely on protection against conventional weapons and on priority ensurance of protection against weapons of mass destruction effects (referred to as WMD). In 1955 the Research Institute of Civil Defense was established. Transfer of civil defense subject to protection against weapons of mass destruction expressed crucial qualitative change that resulted in acceptance of the new Resolution of Government of the Czechoslovak Republic No. 49/1958 Coll. on civil defense of the Czechoslovak Republic. For its support the following services were founded: medical, energy, gas, transport, fire, order, road and bridge, municipal, shelter, water-technical, agricultural, camouflage, construction-technical and supply ones. Operative control of civil defense was ensured by CD staffs whose members were mainly regular soldiers.

Based on the legal resolution of Federal Assembly No. 17/1976 Coll. drawing on doctrinal theory of Warsaw pact countries the subordination of CD was transferred from the Federal Ministry of Interior to the Federal Ministry of National Defense effective January 1, 1976. The State Defense Council became the supreme body for state defense control. Federal Ministry of National Defense was entrusted to become the central body of state administration for organization, coordination and control of CD on the whole area of the

Czechoslovak Republic. Local authorities at all levels were responsible for provision of tasks and needs of CD. Between 1976 and 1989 CD followed directives of the State Defense Council.

In June 1991 the State Defense Council approved the Conception of Civil Defense in the Czech and Slovak Federative Republic reflecting trends of CD development abroad with the aim to focus on protection of population against non-military emergency events. For the first time in the Czechoslovak history clear differentiation of functions and tasks of CD in times of peace and during military alerts was expressed.

Substantial changes were implemented after division of the federal state into two independent ones and after the establishment of the Czech Republic on January 1, 1993. Effective that day the operation of the former Federal CD staff was transferred to the Staff of Civil Defense of the Czech Republic. On March 17, 1993 the Resolution of the Government of the Czech Republic No. 126/1993 Coll. on the state of civil protection in the Czech Republic, its structure and material needs ensurance was passed. The government states there that the formation of the new civil protection system (referred to as CP) will be realized together with the new conception of the Army of the Czech Republic with full respect to the Protocols Additional I and II to the Geneva Conventions. On September 1, 1993 the Central Body for Civil Protection of the Czech Republic was established by the directive of then minister of defense. The body became an authority of the Ministry of Defense responsible for execution of state administration in CP affairs and at the same time it replaced the Staff of Civil Protection of the Czech Republic.

In 1997 the Government of the Czech Republic confirmed the resolution No. 710/1997 Coll. transferring the authority to execute state administration on CP from the Ministry of Defense to the Ministry of Interior and set the effective date on January 1, 2000 in the resolution No. 53/1999 Coll. In the new organization structure the Central Body for Civil Protection of the Czech Republic and regional CP bodies were united with the Fire Rescue Service of the Czech Republic (referred to as FRS). This way the activities of CP of the Czech Republic as an institution was terminated and the General Directorate of FRS of the Ministry of Interior became central authority entrusted with civil protection.

Effective Legislation Focused on Ensurance of Civil Protection

The government of the Czech Republic approved the Conception of Civil Protection until 2006 with the Prospect to 2015 (referred to as conception 2006) in its resolution No. 417/2002. The conception solves protection of civilians systematically. The government obliged ministers and heads of other authorities, governors and city mayors of Prague, Brno, Ostrava and Plzeň to implement fully the measures comprised in the above conception 2006 that emphasizes responsibility of ministries, central state authorities, local authorities, persons and physical persons for CP specified by laws. The conception was revised by the resolution of the Czech government No. 21/2005 Coll.

In the above conception 2006 civil protection was characterized as a system of activities and procedures, subject related authorities, other subjects and individual citizens resulting in minimization of impacts of emergencies on lives and health of inhabitants, on possessions and environment.

At the beginning of 2008 (on February 25) the Government of the Czech Republic passed the resolution No. 165 "On Evaluation of the State of Implementation of the Conception of Civil Protection until 2006 with the Prospect to 2015 and on the Conception of Civil Protection until 2013 with the Prospect to 2020" giving more details on CP in the Czech Republic. This resolution contains 25 pages of specialized text from the field of CP and the Schedule of Implementation of Provisions on CP until 2013 with the Prospect to 2020 (referred to as conception 2013 and schedule). Inspite of the attempt to design the conception 2013 and schedule from the contemporary point of view it is necessary to point at inconsistent solutions of some problems concerned.

Civil protection represents an extremely important aspect of life of modern society. The supreme legislative regulation of the Czech Republic, the Constitution, ensures protection of lives, health, possessions of Czech citizens and protection of environment. Similarly to other developed European countries the security-political situation in the Czech Republic has been re-evaluated during the last decade of the 20th century. As the result of substantial political changes at the beginning of 90-ties military threat in Europe and global war conflict became less probable. Attention was focused on non-military risks important from the point of view of individual countries and security of their citizens.

Regarding the importance of civil protection its problems should be specified and expressed in a special law. Possible content and extent of the suggested law on civil protection follows:

- Introductory provisions
- Definitions and basic terms
- General provisions
- Emergency events and crisis states
- Basic organization and technical measures for civil protection
- Protection of citizens against impacts and effects of emergency events and crisis states
- Preparedness of citizens for emergency events and crisis states
- Execution of state administration in the field of civil protection
- Effectiveness of the act

Besides the above mentioned legislative regulation a project "Who is Who in Civil Protection" should be implemented. Authors and proposers will appreciate inspiring and constructive ideas and comments concerning the project.

Project: Who is Who in Civil Protection in the Czech Republic

Aim of the project:

- to compile a database of specialists in the fields related to civil protection in the Czech Republic
- to support preparedness and capacity of action of professional and voluntary bodies related to civil protection
- to list bodies or people in the database based on voluntary principle (protection of personal data of listed participants of the project)

Project outcomes:

- Proceedings (printed publication)
- Implementation and update of a website

Participating organizations – initial suggestion:

- Universities: e.g. Technical University of Ostrava – Faculty of Safety Engineering; University of Defense Brno – Institute of NBC Defense Vyškov; Police Academy of the Czech Republic, Prague, Department of Crisis Management; Brno University of Technology, Faculty of Chemistry; Tomas Bata University Zlín; University of South Bohemia, České Budějovice; Palacky University Olomouc; University of Pardubice; University of Economics, Prague etc.
- Research institutes and specialized institutions: e.g. Population Protection Institute Lázně Bohdaneč; Military Technical Institute of Protection Brno; National Institute for Nuclear, Chemical and Biological Protection Kamenná; State Office for Nuclear Safety, Prague etc.
- Companies and firms manufacturing and marketing materials for civil protection (e.g. syndicate of companies Czech NBC team etc.)
- Ministries: Ministry of Interior – General Directorate of Fire Rescue Service and Police of the Czech Republic; Ministry of Health – Department of Crisis Preparedness, Emergency Medical Service, Air Rescue Service, selected hospitals; Ministry of Defense; Ministry of Industry and Trade; Ministry of Transport; Ministry of Education, Youth and Sport; Ministry of Environment; voluntary bodies, e.g. Czech Red Cross, Mountain Rescue Service of the Czech Republic etc.

Proposed personal data of specialists to be listed in the "Who is Who in Civil Protection in the Czech Republic" database:

- First name and surname, academical degrees
- Employment history/accomplishments
- Major contemporary professional orientation
- Major supervised projects and publications in the field of CP in recent 5 (10) years
- Contact information for communication: address, telephone and fax numbers, e-mail address etc.

Implementation of the above process makes sense when its outcomes are accessible e.g. on a special website available for selected specialists assigned with passwords and when the database is regularly updated.

Conclusions

Contradictory and contrary character of civilization activities accompanied with permanent proliferation of security risks results in increasing danger for citizens caused by growing number and types of emergency events. The development of corresponding security system lags behind the above described process, which makes the problems of civil protection permanently unresolved.

Civil protection as a system of specialized measures remains an integral part of crisis management and represents its priority in non-military emergency events. Concerning the systematic approach to solve individual types and kinds of emergency events civil protection is understood as a separately controlled and coordinated domain.

With regard to growing importance of civil protection nowadays and in future preparation of and negotiation on special legislative regulations (see the proposed law on civil protection above) based on a "set of acts on crisis states" passed in the half of 2000 year and effective January 1, 2001 can be highly recommended. The field of civil protection deserves proper background in thorough legislation.

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P48 APPLICATION OF DGT METHOD FOR ASSESMENT OF AVAILABILITY OF HEAVY METALS TO PLANTS

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Introduction

At present time problems of toxic metal contamination of soils are often solved. The form of metals present in the environment is an important factor affecting their bioavailability. Metals are often bound to various organic complexing ligands as humic substances, which influences their mobility. Therefore, studies of metal speciation are necessary for understanding how metals can move in nature systems.

Leaching procedures are especially used for determination of toxic metals concentration in soils. Simple leaching procedures using aqua regia, ethylenediaminetetraacetic acid (EDTA), nitric acid or sodium nitrate are usually recommended and applied. However, leaching procedures give no information about the metal fraction which is really available for the root system of plants. Therefore, new approaches are still being searched to obtain a better characterization of bioavailable forms of metals and their transport in soils.

Recently an *in situ* technique capable of quantitatively measuring labile metal species has been developed[1]. This technique, known as diffusive gradients in thin films (DGT), has been successfully used to measure the *in situ* concentrations of metals in natural waters, sediments and soils and has been shown to be a promising tool to assess metal phytoavailability in a wide range of soils. The technique is based on accumulation of solutes in a resin layer after passing through a well-defined diffusive gel layer. The mass of solutes accumulated in the resin during a period of deployment time is measured.

The aim of this work was to assess the heavy metal uptake of radish and to test the capability of DGT to predict phytoavailability of the metals for this plant.

Experimental

Soil Treatment and Leaching Procedures

Homogenized and sieved soil, which had been sampled in Zabcice site, was used in the experiment. Content of Cd and Cu extractable with nitric acid, acetic acid, EDTA, sodium nitrate and water was determined in the soil according to the recommendation of the Community of Bureau of Reference (BCR)[2] and Gupta[3]. Portion of 6 kg of the soil was weight into each of 40 pots. Soil portions in individual pots were spiked by adding solution of Cd and Cu, so that the concentration of the metal in the soil was increased by 1 ppm Cd, 2 ppm Cd, 100 ppm Cu and 200 ppm Cu. The pots with non-spiked soil portions served as control samples. After 3 months, leaching with the same agents was carried out with artificially contaminated samples.

DGT Experiment

The gels for DGT were prepared according to the conventional procedures (DGT Research, Ltd., Lancaster, UK)[4]. The DGT piston probes were deployed at 24 ± 1 °C in each soil sample in triplicate with the moisture content of 150 % of maximum water holding capacity MWHC for 24 hours. After elution of the resin gel with 1M HNO_3, the accumulated mass of Cd or Cu was determined.

Pot Experiment

Radish (*Raphanus sativa*) was sown both in the control and contaminated soils. Five plants of radish were grown in each of four pots with the same soil sample. Six weeks after sowing the radishes were harvested, rinsed with deionized water; the root divided into the white inner part and the red outer part and digested using the dry mode mineralizer (APION). Dry matter content in both parts was determined by drying samples in a oven at 105 °C.

Determination of Metals

Content of Cu and Cd was determined by electrothermal atomic absorption spectrometry (ETAAS) employing Perkin-Elmer Model 4110 Zeeman atomic absorption spectrometer. Recommended conditions were applied.

Results

Concentration of Cd and Cu found in soil samples by leaching with $NaNO_3$ and water and in dry matter of radish edible parts are summarized in Tables I and II. The test sample was also characterized by other leaching agents. Content of elements in this soil related to nitric acid, acetic acid and EDTA leachate fractions was 49.1 ± 2.8; 45.5 ± 1.9; 11.8 ± 0.8 μg kg^{-1} Cd and 6.80 ± 0.50; 0.256 ± 0.051; 3.10 ± 0.17 mg kg^{-1} Cu.

The content of both metals is significantly higher in contaminated soils as intended. The results show that added metals are strongly bound to the soil matrix. The amount of extractable Cd and Cu with $NaNO_3$ reaches only 10 % and even only 0.1 % of added metal, respectively. The concentrations found by means of DGT technique are within 1–2 orders of magnitude lower than the concentrations in sodium nitrate leachates for both metals.

The dry matter content was determined in both analyzed parts of radishes. The red outer part and the white inner part contained on average 11 % and 5 % of dry matter, respectively. Cd and Cu concentration in both parts of radish increases with increasing content of these metals in soils. The Cd and Cu uptake in red outer part of radish is higher than

in white inner part. The fluctuation of individual results indicates higher biological variability among the plants grown in the experimental pots.

Table I
Cadmium content in soils and in dry matter of plant samples grown in these soils

	Concentration of Cd [μg kg^{-1}]		
	Control soil	Cd 1 ppm soil	Cd 2 ppm soil
Leaching – $NaNO_3$	0.47 ± 0.10	46.7 ± 8.5	249 ± 32
Leaching – water	0.85 ± 0.10	1.89 ± 0.73	3.77 ± 0.51
DGT unit	0.015 ± 0.003	1.54 ± 0.26	3.38 ± 0.96
Radish – white part	86.0 ± 20.0	2,430 ± 212	4,860 ± 1,230
Radish – red part	282 ± 67	3,543 ± 762	6,260 ± 1,420

Conclusions

Metal concentration (Cd, Cu) in radish depends on the concentration of the metal in soils in which the plants were grown. DGT technique can provide relevant information on accessible form of elements in the soil.

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Table II
Copper content in soils and in dry matter of plant samples grown in these soils

	Concentration of Cd [μg kg^{-1}]		
	Control soil	Cu 100 ppm soil	Cu 200 ppm soil
Leaching – $NaNO_3$	10.9 ± 2.1	210 ± 11	202 ± 39
Leaching – water	7.71 ± 0.40	75.9 ± 18.9	77.9 ± 6.3
DGT unit	0.191 ± 0.030	3.71 ± 0.19	4.96 ± 0.33
Radish – white part	2,340 ± 440	7,220 ± 1,160	11,600 ± 4,600
Radish – red part	3,520 ± 505	15,200 ± 1,830	42,800 ± 21,400

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P49 SPECTROPHOTOMETRIC MICRODETERMINATION OF PHOSPHATE BASED ON THE ION ASSOCIATION COMPLEX WITH RHODAMINE B IN WATER

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Introduction

Phosphate may be a serious problem for the ecosystem[1,2] since it is the main reason for the eutrophication of natural surface waters. In the presence of phosphate, a considerable growing of anabaena is observed which release toxins after their extinction. Moreover, the phosphates cause a considerably growing of water plants which consume the oxygen contents in water, and interfere with the aquatic life. The visual spectrophotometry[3,4] often based on the interaction of molybdatophosphate or molybdatovanadato-phosphate with some basic dyes is often used for its determination.[5–8] The formation of an ion associate with the sensitive Rhodamine B (Tetraethylrhodamine) was studied in detail in this paper.

Experimental

Chemicals

All chemicals used were in analytical grade quality.

0.01 mol dm^{-3} standard solution of phosphate was prepared from 0.3402 g KH_2PO_4 (Lachema, Brno, Czech Republic) in 250 ml, previously dried 1h at 130 °C.

0.3 mol dm^{-3} solution of sodium molybdate, (Lachema, Brno, Czech Republic) and 1×10^{-3} mol dm^{-3}, solution of Rhodamine B (Tetraethylrhodamine), (Merck, Darmstadt, SRN) in milli Q water were stock solutions.

Brij 35 (Aldrich, Steinheim, SRN), Triton X 100 (Calbiochem Co., San Diego, USA) and Polyvinylalcohol (PVA) (Sigma, Steinheim, SRN) surfactants were in 1 % wt. aqueous solutions.

Astasol standard solutions with 1 g dm^{-3} of SiO_3^{2-}, Ca^{2+}, Al^{3+}, Fe^{3+}, K^+, Na^+, Mg^{2+}, As^{3+}, Cl^-, SO_4^{2-}, NH_4^+, NO_3^-, NO_2^- (Analytika, Praha, ČR), were used for studying interferences.

Real Waters Samples

Surface water from the River Sázava, mineral water Korunní, drinking water from the Brno water supply and sea water from the Mediterranean Sea were sampled. The water samples were filtered by using membrane filter with pore size 0.45 μm.

Instrument

Spectrophotometer Spectronic UNICAM UV 500 (Spectronic Unicam, UK, Cambridge).

Calibration plots and limits of detection

All linear calibration plots were evaluated according to the standard ČSN ISO 8466-1[10] characterizing necessary statistical characteristics for evaluation of linear calibration plots (variation range homogeneity test and linearity test).

The detection limits were expressed according to Graham[9], Miller[11] and to IUPAC[13].

The method of continuous variation[12] was used for the evaluation of the mol ratio of components.

Results

The sequence of mixing components has an important effect for the sensitivity and reproducibility of the method. The maximal absorbance was reached for the following order of mixied components: phosphate → nonionic surfactant → sodium molybdate → sulphuric acid → Rhodamine B. The absorbance of the ternary species of 12–molybdatophosphate with Rhodamine B reaches its maximum value for 8.3×10^{-5} mol dm^{-3} Rhodamine B and 0.03 mol dm^{-3} sodium molybdate after 20 min. at 572 nm. The higher concentrations of both components are responsible for the absorbance decreases.

Effect of Acidity

In the following range 0.1–3.0 mol dm^{-3}, the absorbance considerably decreased with the increasing concentration of sulphuric acid. 1 mol dm^{-3} of H_2SO_4 was optimal for obtaining stable absorbance of the associate in time. Hydrochloric acid has a similar effect but 1.5 mol dm^{-3} was used for further measurements.

Effect of surfactants

Three non-ionic surfactants were used, Brij 35, Triton X 100 and PVA respectively, from which 0.01 % wt. of Brij 35 was optimal. In the presence of Triton X 100 the adsorption of the ion associate on glass surface was observed. PVA 30,000–70,000 mol. weight does not prevent turbidity in solution.

Calibration Plots and Detection limits (LOD)

The strictly linear calibration plots were evaluated for six concentration levels between 1×10^{-6}–7×10^{-6} mol dm^{-3}. The points were measured in triplicate for the optimal conditions 1 mol dm^{-3} sulphuric acid, 0.01 % wt. Brij 35, 0.03 mol dm^{-3} sodium molybdate and 8.3×10^{-5} mol dm^{-3} Rhodamine B for the evaluation were used.

Effect of ions

1,000 : 1 Mg^{2+}, K^+, Na^+, NH_4^+, NO_3^-, SO_4^{2-}, Cl^-, HCO_3^- did not interfere the determination of 1×10^{-6} mol dm^{-3} H_2PO_4 and 100 : 1 Al^{3+}, Fe^{2+}, Ca^{2+}. As (III, V) and NO_2^- interfered above concentrations only which are not present in natural waters. The SiO_3^{2-} was successfully masked with 6.7×10^{-4} mol dm^{-3} tartaric acid.

Table I
Calculated values from calibration plots

$y = 0.1421x + 0.0104$		$R^2 = 0.9987$
$X_{\Delta}^{\alpha\,a}$	0.40×10^{-6} mol dm^{-3}	1.24×10^{-5} g dm^{-3}
$X_{\Delta}^{\beta\,a}$	1.18×10^{-6} mol dm^{-3}	3.66×10^{-5} g dm^{-3}
$X_{m}^{\,b}$	0.79×10^{-6} mol dm^{-3}	2.44×10^{-5} g dm^{-3}
$X_{3\sigma}^{\,c}$	0.83×10^{-6} mol dm^{-3}	2.56×10^{-5} g dm^{-3}
$\varepsilon_{\sigma}^{\,d}$	142123 ± 3767	mol^{-1} cm^{-1} dm^3

The Stoichiometry of the Ternary Species of 12-Molybdatophosphate with Rhodamine B

The molar ratio of components in the ion associate was expressed from the method of continuous variations12.

$n = \frac{x_{max}}{1 - x_{max}}$. The resulting value of n was $n = \frac{0,5}{1 - 0,5} = 1$.

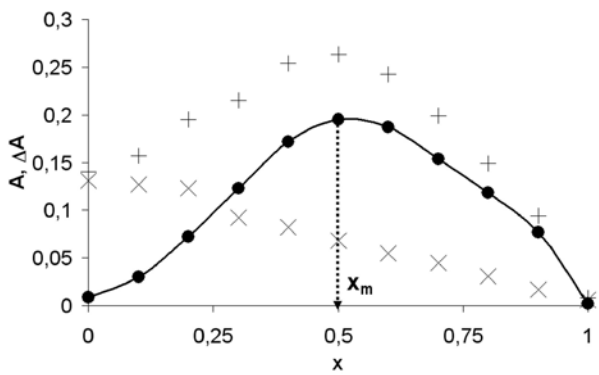

Fig. 1. **The point 0 on the Jobs curve corresponds with the maximal concentration of the Rhodamine B and zero concentration of phosphate, the point 1 with the maximal concentration of phosphate and zero concentration of Rhodamine B. + points belong to the total absorbance of solution, × points belong to the Rhodamine B blank**

This molar ratio corresponds with the ratio between phosphate and Rhodamine B as 1 : 1. Which describes the composition of the ion associate such as $H_2P[Mo_3O_{10}]_4^- \cdot RhB^+$ in 1 mol dm^{-3} H_2SO_4.

Applications For the Real Samples of Water

For all kinds of water, the method of standard additions in six concentration levels which are 1, 2, 4, 6, 7×10^{-6} mol dm^{-3} was used. The concentration of phosphate was found 0.1 ± 0.02 mg dm^{-3} triplicate the values in the river water and 0.16 ± 0.007 mg dm^{-3} phosphate in the drinking water. The determination could not be carried out by this approach for mineral water because of increased contents of silicate in solution. The optimal concentration of 6.7×10^{-4} mol dm^{-3} tartaric acid is not effective for masking but the necessary higher concentration of tartaric acid would interfere with the determination of phosphate. The concentration of phosphate in the analyzed sea water was under the detection limit and the method of standard addition can not be used.

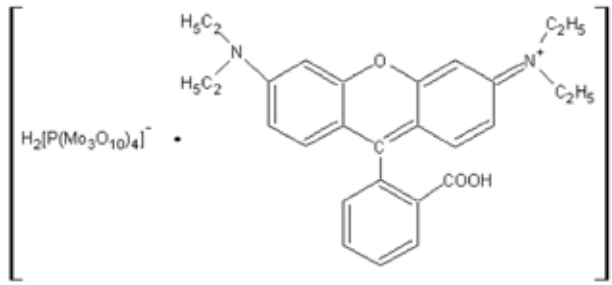

Fig. 2. **The supposed structure of the ion associate**

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P50 DETERMINATION OF URANIUM BY ICP-AES IN THE ABSENCE AND PRESENCE OF PRECONCENTRATION ON MACROPOROUS SORBENTS

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Introduction

Determination of uranium at very low concentrations often needs preconcentration in order to meet the detection limit of a given analytical method. Matrix interferences are another problem when using AAS and ICP-AES. The preconcentration by solid phase extraction is simple, rapid and usually help to eliminate interferences from the matrix elements. Several sorbents were used for preconcentration and separation of trace uranium (VI)[1], among them the macroporous Amberlite XAD resins[2,3], silica[4,5], active carbon[6] and polyurethane foam[7] which can be loaded with completing reagents. The modified Amberlite XAD 4 resin in various particle size was studied for the sorption of uranium in this paper prior to the final determination by ICP-AES[1–3].

Experimental

Chemicals

All chemicals used were of analytical grade quality.

1 g dm^{-3} standard solution of uranium (VI) Astasol (Analytika, Praha, Czech Republic)

0.5 g dm^{-3} solution of 4-(2-pyridylazo)resorcinol (PAR) from, Lachema, Brno, Czech Republic, the ammonium salt of pyrrollidincarbodithioate (APDC) from, Lachema, Brno, Czech Republic, 8-hydroxyquinoline-5-sulphonic acid (8-HQS) from Aldrich, Steinheim, Germany and 1,2-dihydroxybenzene (PYR) from Lachema, Brno, Czech Republic in distilled water were stock solutions.

Cationic surfactants 1-ethoxycarbonylpentadecyl-trimethylammonium bromide (Septonex®) from Tamda, Oloumouc, Czech Republic, benzyldimethyltetradecyl-ammonium chloride (Zephyramin®) from Merck, Darmstadt, Germany and benzyldimethyldodecyl-ammonium bromide (Ajatin®) from Fluka, Buchs, Switzerland were in 0.1 mol dm^{-3} aqueous solutions.

The macropourous sorbent Amberlite® XAD 4 (Fluka, Buchs, Switzerland) was previously dried 24 h at 100 °C, milled and sieved; the fraction 0.32–0.63 μm was used and ctivated in methanol for 24 hours. 200 mg of activated sorbent was filled into empty cartridges. The columns were finally washed by 10 ml of acetone and 10 ml of distilled water.

Solutions for the sorption or the eluent were aspirated through the sorbent-filled plastic cartridges using the vacuum pump operated vacuum suction device Dorcus™ (Tessek, Praha, Czech Republic). A peristaltic pump UNIPAM 315™ (Scientific instrument, Warszawa, Poland) was attached with 3 mm wide silicon tubing to the cartridges and operated at a solution flow rate of 1 ml min^{-1}.

Instrument

An echelle-based ICP-spectrometer with a prism predisperser IRIS AP™ (Thermo Jarell Ash, U.S.A.) containing a CID detector with 512 × 512 pixels for 195–900 nm, axial plasma discharge and echelle grating with 54.4 lines mm^{-1} was used. The plasma source was a generator with 27.12 MHz with the power output of 1.35 kW. The plasma argon flow rate was 12 dm^3 min^{-1}. The integration time was 30 s. The results were the average of 3 measurements. Spectral lines (nm) in high orders: U 385.958 and U 409.014 nm were tested for the determination and the spectral line 385.958 was used for future measurement.

Calibration Plots and Limits of Detection

All linear calibration plots were evaluated according to the ČSN ISO 8466-1 standard including the variation range homogeneity test and linearity test[8]. The confidence limits of the plot are also expressed.

The recovery was calculated by the expression

$$R = \frac{c(U)_{eluted}}{c(U)_{applied\ onto\ the\ column}} \cdot f \qquad (1)$$

where f is the enrichment factor.

The detection limit was expressed according to Graham[9], Miller[10] from the calibration plots and to IUPAC[11] from 10 points of the blank.

Results

Determination of Uranium by ICP-AES in the Absence of Preconcentration

Some determination in geological samples was earlier described[12]. A 10% signal decrease was observed for 0.75 mol dm^{-3} HNO_3, but for 1 mol dm^{-3} HCl the decrease reaches 20 %. In the presence of various 3×10^{-3} mol dm^{-3} surfactants, Brij 35, Zephyramine, Ajatin and dodecylsuphate the 5–15 % increase of the calibration slope was observed. Similarly, the slope of calibration plots increased by 5 % in the presence of 6×10^{-5} mol dm^{-3} PAR, 9×10^{-5} mol dm^{-3} APDC or 2.2×10^{-5} mol dm^{-3} 8-HQS. (cf. Table I)

Calibration plots and effect of interfering ions

The strictly linear calibration plots were evaluated for seven concentration levels between 0.1 mg dm^{-3}–50 mg dm^{-3}. The points were measured in triplicate. The detection limits were $X_\Delta{}^\alpha$ = 1.26 mg dm^{-3}, $X_\Delta{}^\beta$ = 3.69 mg dm^{-3} according to Graham, $X_\Delta{}^m$ = 1.13 mg dm^{-3} according to Miller and 0.30 mg dm^{-3} according to IUPAC.

For 1–3 mg dm^{-3} U, no interference were observed for 100 : 1 NO_3^-, Cl^-, SO_4^{2-}, SiO_3^{2-}, NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+},

Table I
The regression equation for different selected systems

U (VI) Solution	regression equation
Basic[a]	$y = 4.48x + 1.29$
Septonex[b]	$y = 4.49x + 0.23$
Dodecylsulphate[c]	$y = 5.37x + 1.08$
0.75 mol dm^{-3} HNO3	$y = 4.04x + 2.18$
1 mol dm^{-3} HCl	$y = 3.75x + 3.18$
PAR[d]	$y = 4.95x + 1.32$
APDC[e]	$y = 4.83x + 0.43$

[a]calibration plots for 1 mg dm^{-3}–15 mg dm^{-3} U without organic reagents ;
[b]3×10^{-3} mol dm^{-3} Septonex;
[c]3 × 10-3 mol dm^{-3} sodium dodecylsulphate;
[d]2×10^{-5} mol dm^{-3} PAR;
[e]3×10^{-5} mol dm^{-3} APDCin solution

Al^{3+}. The 10 : 1 excess of multicomponent standards containing Br^-, Cl^-, Br^-, SO_4^{2-}, PO_4^{3-} or Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Mn^{2+}, Pb^{2+}, V^{5+} and Zn^{2+} (each of metal ion) in the absence or presence of Y^{3+} internal standard (1 mg dm^{-3}) did not interfere. 10 : 1 excess of Fe^{3+} interferes strongly by the 50% signal decrease in the presence of Y^{3+}.

Determination of Uranium by ICP-AES After the Preconcentration on Modified Amberlite XAD 4

Prior to the sorption, the column was conditioned by 10 ml of 5×10^{-3} mol dm^{-3} Septonex whose pH was adjusted by hydrochloric acid and sodium hydroxide. This procedure was always used for the sorption.

Effect of Surfactants and Organic Reagents

The retention efficiency of 3 mg dm^{-3} U from 50 ml volume with the optimal pH 9 without surfactants and organic reagents was 56 % only. The elution of uranium from the column was carried out with 10 ml acetone and 1 mol dm^{-3} nitric HNO_3 (1 : 1). The various surfactants were used such as non-ionic Brij 35, cationic Septonex or Zephyramine and anionic dodecyl sulphate respectively, from which 5×10^{-3} mol dm^{-3} of Septonex was optimal for conditioning and allowed the recovery of 96 % U. The recovery with dodecylsulphate was 81 % U. Brij 35 produced a foam in the column and Zephyramine did not prevent turbidity during evaporation, when HNO_3 was used for elution.

Moreover the sorption of 1.5–15 mg dm^{-3} of U was quantitative by using 6×10^{-5} mol dm^{-3} PAR and 9×10^{-5} – 1.8×10^{-4} mol dm^{-3} APDC which corresponds with the 5 or 10 fold excess of reagent and the recovery nearly 100 % was obtained in the presence of 1.3×10^{-4} mol dm^{-3} 8-HQS and 1.4×10^{-4} mol dm^{-3} PYR. Thus, 6×10^{-5} mol dm^{-3} of PAR was recommended for following preconcentration.

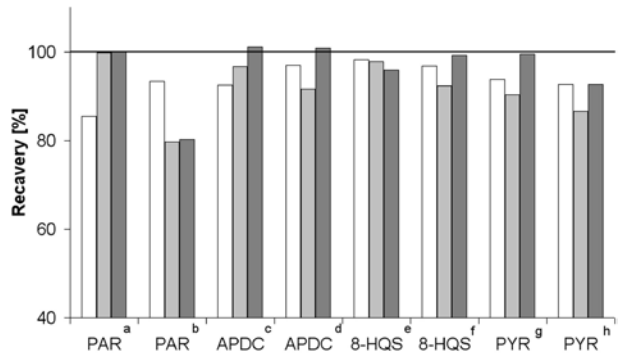

Fig. 1. **Effect of organic reagents. □ pH = 5; ■ pH = 7; ■ pH = 9; [a]6×10^{-5} mol dm^{-3} PAR, [b]1.2×10^{-4} mol dm^{-3}PAR, [c]9×10^{-5} mol dm^{-3} APDC, [d]1.8×10^{-4} mol dm^{-3} APDC, [e]6.7×10^{-5} mol dm^{-3} 8-HQS, [f]1.3×10^{-4} mol dm^{-3} 8-HQS, [g]1.4×10^{-4} mol dm^{-3} PYR; [h]2.7×10^{-4} mol dm^{-3} PYR, 3 mg dm^{-3}U was sorbed after conditioning the sorbent with 5×10^{-3} mol dm^{-3} Septonex solution**

Effect of pH

The influence of pH on the sorption follows from Table II. The sorption was quantitative in the interval of pH 7–9.

Table II
Effect of pH on the sorption[a]

pH	recovery [%]
9	100.0 ± 3.6
8	99.5 ± 3.7
7	100.6 ± 2.4
6	89.6 ± 4.8
5	88.6 ± 5.3
4	82.7 ± 0.9

[a]From 50 ml of sample with 3 mg dm^{-3} U, in the presence of 6×10^{-5} mol dm^{-3} PAR and after conditioning the sorbent with 5×10^{-3} mol dm^{-3} Septonex solution. The sorption was carried out in triplicate

Effect of Eluents

For the quantitative elution of the complex of uranium (VI) with the used reagents, acetone, ethanol and methanol in different ratio with 1 mol dm^{-3} nitric acid were tested. The most effective eluent with 100% recovery of U was 10 ml of 1 : 1 acetone and 1 mol dm^{-3} nitric acid or the 1 : 1 mixture of acetone and 4 mol dm^{-3} HNO_3 mixtures. The best eluents are compared in Table III.

Conclusions

The sorbent XAD-4 in the presence of 4-(2-pyridylazo)resorcinol or pyrrollidincarbodithioate was observed for the separation and preconcentration of Uranium (VI) at pH 9. The column of Amberlite XAD 4 of particle size 0.32–0.63 μm was washed with 10 ml of acetone and 10 ml of distilled water, and conditioned with

Table III
The recovery with different eluents

Elution reagent	Recovery of U [%][a]
Ethanol-HNO_3 [1 mol dm^{-3}]	92 ± 5
Aceton-HNO_3 [1 mol dm^{-3}]	100 ± 3
Methanol-HNO_3 [1 mol dm^{-3}]	94 ± 2

[a]eluted with 10 ml of eluent in triplicate

5×10^{-3} mol dm^{-3} Septonex solution. The sample solution containing 1.5–15 mg dm^{-3} U with 6×10^{-5} mol dm^{-3} of PAR was applied on the column with 1 ml min^{-1}. The column was then washed with 15 ml of distilled water and uranium eluted with a mixture of acetone : 1 mol dm^{-3} HNO_3 (1 : 1) . The organic solvent was removed by evaporation under an IR lamp to 2 ml in a suitable Teflon® dish. The residue was then diluted to 10 ml by distilled water and analyzed by ICP-AES. (cf. Table IV)

Table IV
The efficiency for uranium (VI) in the presence of org. reagent at pH 9 on Amberlite XAD 4

Org. reagent	Uranium (VI) 1.5 mg dm^{-3}	3 mg dm^{-3}	15 mg dm^{-3}
PAR[a]	1.53 ± 0.02	2.99 ± 0.05	14.75 ± 0.12
APDC[b]	1.65 ± 0.09	3.05 ± 0.05	15.69 ± 0.15
APDC[c]	1.43 ± 0.07	3.01 ± 0.05	14.48 ± 0.69

[a]6×10^{-5} mol dm^{-3} PAR,
[b]9×10^{-5} mol dm^{-3} APDC,
[c]1.8×10^{-4} mol dm^{-3} APDC,
[d]The sorbent conditioned with 5×10^{-3} mol dm^{-3} Septonex and the sorption carried out in triplicate

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P51 SIMULTANEOUS SPECIATION OF SELENIUM AND MERCURY IN ENVIRONMENTAL SAMPLES BY USING A COLUMN SWITCHING SYSTEM WITH LIQUID CHROMATOGRAPHY COUPLED TO ICP-MS

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Introduction

Mercury is a very toxic element which damages the central nervous system, endocrine system, kidneys, and other organs. Exposure over long periods of time or heavy exposure to mercury vapour can result in brain damage and ultimately death. Mercury and its compounds can produce serious birth defects.Compounds of mercury tend to be much more toxic than the element itself.[1–3]

On the other hand selenium is an essential micronutrient for animals with biological functions as cofactor. However it is toxic in large doses. Selenium deficiency can lead to Keshan and Kashin-Beck diseases. If it is taken in excess it can lead to seleniosis. In addition, several studies have suggested a link between cancer and selenium deficiency[4].

It has been issued that Se-methionine inhibits some neurotoxic effects of methylmercury.[5–7]

For this reason, the development of new analytical strategies for multielemental speciation is a primordial issue.

In the present study, a new method for the detection of Se- and Hg- species has been developed, including chiral species.

Experimental

Instrumentation

The HPLC system is an Agilent 1100 series. The columns used were a Phenomenex Bondclone C18, 300 mm × 3.90 mm, 10 μm; and an Astec Chirobiotic T column, 250 mm × 4.6 mm.

An inductively coupled plasma mass spectrometer Model HP 4500 (Hewlett Packard, Yokogawa, Analytical System, Tokyo, Japan) equipped with a Babington nebuliser was used in this study.

Reagents and Standards

All reagents were of analytical reagent grade. Deionized water (18 MΩ cm^{-1}) was obtained from a Milli-Q water purification system (Millipore, UK). 2-mercaptoethanol 98% was purchased from Sigma–Aldrich (Steinheim, Germany) and tetraethylammonium chloride from Fluka (Switzerland). Ammonium acetate and nitric acid were obtained from Merck (Darmstadt, Germany).

Stock standard solutions of 1,000 mg Se dm^{-3} were prepared in deionized water from selenocystine ($SeCys_2$, Sigma), seleno-DL-methionine (Se-DL-Met, Sigma), seleno-L-methionine (Se-L-Met, Sigma), selenomethylselenocysteine (SeMeSeCys, Sigma), selenocystamine (SeCA, Sigma), sodium selenate (Na_2SeO_4) and sodium selenite (Na_2SeO_3).

Methylmercury chloride stock standard solution was prepared at 1,000 mg Hg dm^{-3} by dissolving methylmercury chloride (Merck (Darmstadt, Germany) into 2% HNO_3. Mercury chloride stock standard solution was prepared as 1,000 mg Hg dm^{-3} solution by dissolving mercury chloride (Merck (Darmstadt, Germany) into 10% HNO_3.

Procedure

A 0.075% tetraethylammonium chloride water solution at pH 4.5 (mobile phase A) and a 5% (v/v) methanol-water solution containing 0.06 mol dm^{-3} ammonium acetate and 0.1% (v/v) 2-mercaptoethanol (mobile phase B) were used as the mobile phases for HPLC. The flow rate was 1 ml min^{-1} and the sample injection volume was 100 μl. The columns were connected using three valves to build a column swithching system and species were on-line detected by ICP-MS. The columns outlets were connected directily to the nebulizer of the ICP-MS system.

Elemental detection was performed using a model 4500 ICP-MS system. The plasma and auxiliary argon flow rates were 15 and 1 dm^3 min^{-1}, respectively. The nebulizer gas flow rate was 1.28 dm^3 min^{-1}. The forward RF power was fixed at 1,266 W. The dwell time was 3 seconds per isotope and ^{77}Se, ^{82}Se and ^{202}Hg were monitored.

Table I
HPLC conditions

Time	Mobile phase	Columns
0–5.1	A	RP
5.1–6.15	A	RP + Chiral
6.15–8.3	A	RP
8.3–13.3	A	RP + Chiral
13.3–25	B	RP

RP = Phenomenex Bondclone C18 column,
300 mm × 3.90 mm, 10 μm
Chiral = Astec Chirobiotic T column, 250 mm × 4.6 mm,

In our study of selenium and mercury speciation have been successfully separated six selenium compounds and two major mercury compounds in biological samples using a reversed-phase column. The chiral species of selenium was later separated with the second column.

After the injection in the loop, all species go into the reversed phase column and later directly to the ICP-MS, using the mobile phase A. With this program, SeCM, SeCys, SeMeSeCys and Se (IV) elute before 5.1 minutes. At 5.1 minutes we active the second column, and D-selenomethionine and L-selenomethionine go throught the chiral column. After that, at 6.15 minutes we switch off the chiral column,

then Se (VI) go out from reversed phase column to the ICP-MS. At 8.3 minutes we switch on the chiral column again going out D-Selenomethionine and L-Selenomethionine. At this moment, MeHg and inorganic mercury are still inside the reversed phase column and to get their separation, at 13.3 minutes we disconnect the chiral column again and the mobile phase B is pumped. With this change we get the elution of MeHg and inorganic mercury.

Results

Fig. 1. illustrates the chromatograms obtained with the method proposed and the Table II show the species detected, their retention times, detection limits and linear range. The detection limits vary between 0.3 and 9.7 ng depending of the species. The Relative Standard Deviation (% RSD) for the retention time is below 1 % for all species and for peak area is below 19 % for all species.

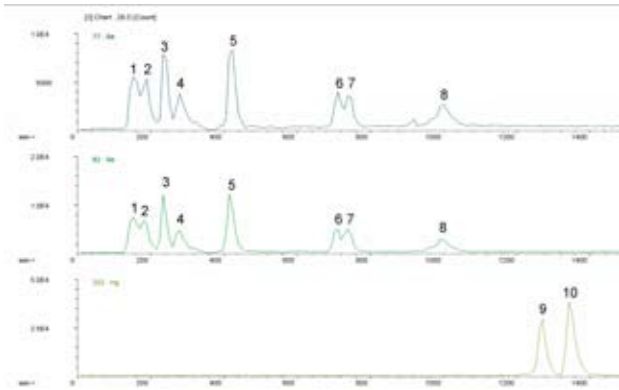

Fig. 1. **Chromatogram obtained by the proposed method for ^{77}Se, ^{82}Se and ^{202}Hg isotopes**

Conclusions

This work reports for the first time an analytical methodology for the chromatographic separation of mercury and selenium species including chiral ones.

The methodology proposed for the simultaneous speciation allows a deeper insight into the interaction between Se and Hg-species which is a key question due to the beneficious effect of Se-species into Hg toxicity.

Table II
Retention times, detection limits and linear range of the species

Peak	Species	Retention time [s]	Detection limit [ng]	Linear range [ppb]
1	Se-cystamine	162.2	6.24	62.4–1,000
2	Se-cystine	189.2	4.03	40.2–500
3	Se-methyl-selenocysteine	243.3	7.78	77.8–1,000
4	Se (IV)	279.3	7.26	72.6–1,000
5	Se (VI)	420.8	6.63	66.3–1,000
6	Se-L-methionine	717.3	9.67	96.7–1,000
7	Se-D-methionine	752.4	4.78	47.8–1,000
8	Peak due to mobile phase change			
9	Methylmercury	1,265.1	4.40	44.0–10,000
10	Inorganic mercury	1,344.4	0.30	3–10,000

The developed methodology allows high sample throughput and low sample consumption that is highly important for the application to food and biological samples.

This method don't has memory effect in the system.

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P53 VOLATILE ORGANIC SUBSTANCES PRESENT IN SPICES AND SPRUCE NEEDLES

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Introduction

Essential oils are volatile lipophilic substances, usually colorless. Most often, essential oils consist of terpenes, namely monoterpenic hydrocarbons, aldehydes, alcohols, ketones, acids, esters. Their content substances are usually classified as isoprenoids and phenylpropanoids groups[1]. Their characteristic scent is conditioned by terpenic compounds, in general[2].

The TLC (thin layer chromatography) method can be used for the identification of essential oil present in spice[1]. This method is simple, without need of sofisticated and expensive instrumentation. Defined amount of the analysed mixture is applied on the starting line of a plate covered by a thin layer of sorbent (stationary phase). Chromatographic plate is then placed into the developing chamber with mobile phase, which rises slowly and evenly through the thin layer, transporting the individual components of the analyzed mixture by various speed. Dried thin layer with perceptible stains of particular compounds of the mixture situated in different distances from the start – represents TLC chromatogram. The identification of compounds is performed either via comparison of their migration distances with standards, or by comparison of their R_F values with those obtained from the literature[3].

Another option to identify essential oils is application of SPME (Solid phase microextraction) in connection with GC/MS. Solid phase microextraction is simple and efficient sorptive – desorptive technique used for solventless isolation/preconcentration of target analytes from the sample matrix[4]. In the field, this procedure could be also used as passive sampling method. During this procedure, analytes are sorbed by thin layer of stationary phase placed on the SPME fiber. The SPME process continues until the equilibrium in the system is reached. In physical-chemical terms, the SPME technique state of equilibrium depends on the analyte properties and on the type and thickness of polymer covering the silica fiber[4].

Experimental

For the identification of essential oils present in spice (caraway, cardamom, pepper, sweet pepper, calamint, cinnamon and muscat), two methods were used[5]:

- TLC
- SPME, GC/MS

T L C

Spice essential oils isolation proceeded in the following manner. Spice samples were extracted by ethanol for 10 minutes. After that, the extract was filtered and the spice was reextracted twice for 20 minutes by petroleum ether. Extracts were concentrated on the vacuum rotary vaporizer to the defined volume.

By means of micropippete, concentrated extracts were applied on the chromatographic plate (Alugram Sil G). The distance of applied stains was between 0.5–1 cm, the volume of applied sample was always 10 µl and 20 µl. Ethanol and petroleum ether extracts I and II were applied on plates.

Plates were developed in a closed chromatographic chamber, which was filled with a developing agent – mobile phase formed by the mixture of toluene and ethyl acetate (ratio 93 : 7). Developing was ascensive and was let in progress until the mobile phase reached the distance of 1 cm from the top of the plate. Plates were let to dry and then they were sprayed by developer for the purpose of visualization of stains created by separated substances. The used developer consisted of ethanol and sulphuric acid (ratio 95 : 5), which was mixed in 1 : 1 ratio with one-percent solution of vanillin in ethanol. After the chemical detection, plates were dried again in the drier at the temperature of 105 °C for 5 minutes. Identification of vizualized stains was performed via comparison of experimental R_F values with those published in the literature[6].

S P M E – G C / M S

Weighted amounts of individual spices (1 g) were put into vials. Substances from spice were sorbed from the headspace by SPME fiber at the temperature of 40 °C. The compounds were then directly injected into the gas chromatograph.

The SPME fiber used was 65 µm polydimethylsiloxan/divinylbenzene (PDMS/DVB) from Supelco. Gas chromatograph with mass spectrometric detector was Agilent 6890N GC/5973 MSD. The HP-5MS column (Agilent Technologies, USA), 30 m × 0.25 mm × 0.25 µm was used, the injector temperature was 270 °C, oven program was: 45 °C, 2 min, 5 °C to 200 °C, hold 2 min. He at a flow of 1 ml min^{-1} (constant flow mode) was used as a carrier gas, transferline temperature was 250 °C, ion source temperature was 230 °C, quadrupole temperature was set to 150 °C. Direct interface connection was applied, electron ionization at 70 eV was employed.

Results

TLC chromatograms evaluation was performed in accordance with requirements of the Pharmaceutical Codex[6], which is valid for phytopharmaca and recomends TLC as an optimum screening method. R_F values were calculated for each of detected stains. This factor was also used for the identification. Besides the retention factor, also the colours of the stains were compared. For example, the comparison of all sweet pepper extracts shows Table I. In column "Identified Compound", unambiguously identified content substance

is presented in bold; where no compliance existed, "n.i." is stated, which stands for "not identified". Concerning results, it is evident that not all the content substances were present in all extracts. Mostly, they were detected in the ethanolic extract and in the first petroleum extract and their presence in these two extracts was influenced by the chemical nature of these substances.

Table I
Identification of volatile compounds in sweet pepper extracts

RF Ethanol	RF Petroleum ether I	RF Petroleum ether II	Identified compound
–	0.17	–	pinene
0.23	0.22	–	cymene
0.28	–	–	terpinene
0.38	0.41	0.39	n.i.
0.52	0.65	0.67	n.i.
0.69	0.68	–	n.i.
0.93	–	–	n.i.
0.96	–	–	n.i.
0.99	–	–	n.i.

As the second method of essential oils components analysis, SPME in connection with GC/MS was used. The method was optimalized and measurements were performed under conditions mentioned above. Also by this method, not the concentration of substances, but their identification was the matter of concern. In contrast to TLC, the isolation/preconcentration of target compounds was performed via headspace method. To confirm the presence of a given substance NIST spectral library search was used. The Fig. 1. shows chromatogram of sweet pepper spice. In Table II is a summary of substances identified via spectral library search in this spice.

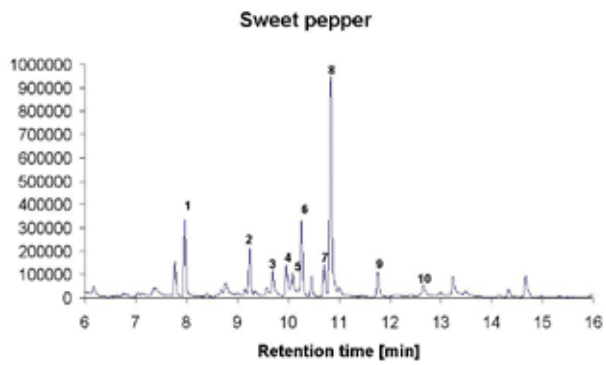

Fig. 1. **Chromatogram of sweet pepper**

By comparison of Tables I and II it is obvious, that more single volatile substances and their isomers can be recognised

Table II
Retention times and identification of compounds present in sweet pepper

Peak Number	Retention Time	Identification
1.	7.97	α-pinene
2.	9.24	β-pinene
3.	9.71	β-myrcene
4.	10.09	α-felandrene
5.	10.26	3-karene
6.	10.46	α-terpinene
7.	10.71	p-cymene
8.	10.84	limonene
9.	11.76	γ-terpinene
10.	12.66	4-karene

by means of SPME in connection with GC/MS. Similar comparison could be made at all spices used.

Conclusion

Analytical separation-based methods were used for the identification of content substances present in essential oils of seven spice species. Following results were obtained:

- The isolation of essential oils content substances can be performed by the means of either appropriate solvent extraction, or passive sampling via SPME.
- Screening chromatography method on the thin layer (TLC) is appropriate for the quick identification of content substances in essential oils. This method is also recommended by the Pharmaceutical Codex[6].
- Decisive GC/MS method enabled the identification of more content substances, including some isomers, at all spice and herbal tea samples analysed.

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P54 CHANGES IN CAROTENOIDS PATTERN IN *MOUGEOTIA SP.* ALGAE INDUCED BY HIGH LIGHT STRESS

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Introduction

When exposure to light exceeds a maximum that can be used productively by the photosynthesys, a violaxanthin de-epoxidation leads to antheraxanthin and finally to zeaxanthin, the excessive energy being then dissipated as heat[1]. At lower irradiance, zeaxanthin is re-epoxidated back to violaxanthin by zeaxanthin-epoxidase (Fig. 1.).

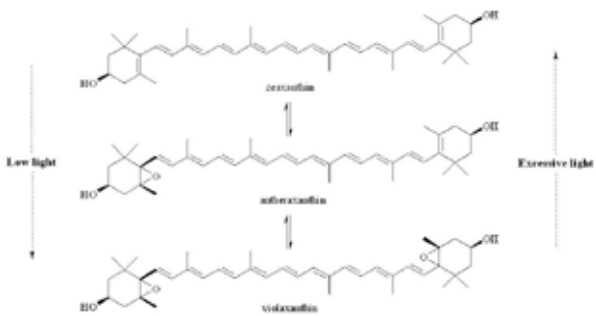

Fig. 1. **The xanthophyll cycle**

This reversible interconversion of zeaxanthin and violaxanthin via antheraxanthin was called xanthophyll cycle or violaxanthin cycle, being initially studied in higher plants[6,7]; further researches established that it has a photoprotective role, removing the excess excitation energy from the photosynthetic antennae[1–4], protecting in this way photosynthetic organisms from dammage by excessive light. The aim of this research was to establish the way in which the carotenoid biosynthesis is influenced by high light stress in the green algae *Mougeotia sp.* Agardt.

Experimental

The carotenoid standards were kindly provided by F. Hoffmann – La Roche, Basel, Switzerland. All solvents were HPLC grade purity (ROMIL Chemicals). The green algae *Mougeotia sp*. Agardt (AICB 560) originated from the collection of the Institute of Biological Researches Cluj-Napoca; it was grown in a Bold nutritive solution mixed by introducing air containing 5 % CO_2, under continuous illumination (300 $\mu mol\, m^{-2}\, s^{-1}$, measured with a Hansatech Quantum Sensor QSPAR), at an average temperature of 20 °C for 15 days. Extraction and high performance liquid chromatography analysis (HPLC) were conducted according to a previous published procedure[5]. Separations were performed on an Agilent 1100 system, using a Nucleosil 120-5 C_{18} column and the following mobile phases: A – acetonitrile : water (9 : 1) and B – ethyl acetate. The flow rate was 1 $ml\, min^{-1}$. and the solvent gradient was as follows: from 0 to 20 min. – 10 % to 70 % B, then from 20 to 30 min. – 70 % to 10 % B.Carotenoids identification was completed based on HPLC co-chromatography with authentic carotenoid standards.

Results

The HPLC chromatogram from Fig. 2.a reveals the carotenoid pattern for the saponified extract of *Mougeotia sp*. control sample, dominated by two major carotenoids: lutein and β-carotene. Besides, four xanthophylls (violaxanthin, lutein, zeaxanthin and 5,6-epoxy-β-carotene) and four caroteneş (α-carotene, β-carotene, 9Z-β-carotene and 15Z-β-carotene) were also identified.

When the *Mougeotia* culture was exposed to a high light irradiation (4,500 $\mu mol\, m^{-2}\, s^{-1}$), the content of antheraxanthin increased strongly as a result of de-epoxidation (Fig. 2.b, Table I), the carotenoid pattern being dominated by lutein and antheraxanthin, while among minor carotenoids 5,6-epoxy-β-carotene moved out and zeaxanthin appeared.

Table I
The carotenoid concentrations of target carotenoids [$\mu g\, ml^{-1}$ algal suspension]

Carotenoids	Control sample	Irradiation with 4,500 [$\mu mol\, m^{-2}\, s^{-1}$]	Recovery after irradiation
Violaxanthin	0.02	0.01	0.10
Antheraxanthin	0.10	0.60	0.05
Lutein	1.00	0.56	0.37
Zeaxanthin	0.00	0.05	0.02
5,6-epoxy-β-carotene	0.04	0.00	0.03
α-carotene	0.04	0.01	0.02
β-carotene	0.19	0.03	0.10
9Z – β-carotene	0.04	0.01	0.02
15Z – β-carotene	0.01	traces	0.01

The whole carotenoid pattern was affected by the light stress (Fig. 2.a and 2.b), not only the xanthophylls involved in the xanthophyll cycle. Chromatograms emphasize another important aspect in the studied matrix: the xanthophyll cycle converts violaxanthin mainly in antheraxanthin, not in zeaxanthin; this finding agrees with results reported for *Mantoniella squamata*[3], where they were attributed as consequences for the mechanism of enhanced non-photochemical energy dissipation. The recovery after the light stress leads to a reversible epoxidation to violaxanthin, revealed by the chromatogram from Fig. 2.c, the final higher violaxanthin level being correlated with a strong decrease in antheraxanthin concentration (Fig. 2.c, Table I), while the new chromatographic pattern is dominated by four major carotenoids: lutein, β-carotene, violaxanthin and 5,6-epoxy-β-carotene.

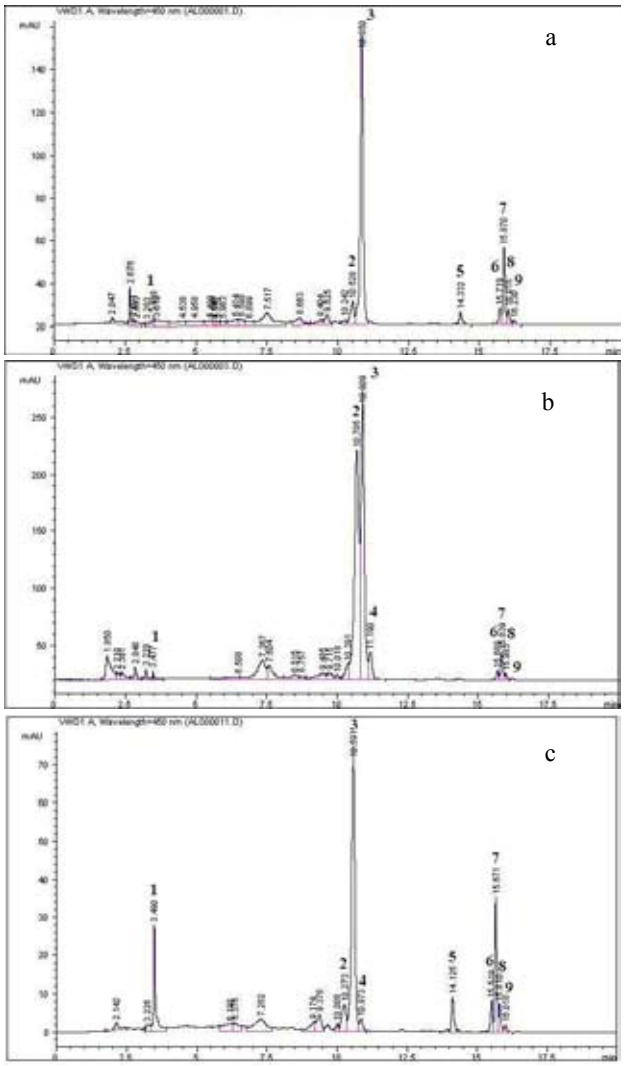

Fig. 2. **HPLC chromatograms of carotenoids from the Mougeotia sp. samples: a – control sample; b – illumination with 4,500 µmol m^{-2} s^{-1}; c – recovery after illumination. Peak identities are: 1: violaxanthin, 2: antheraxanthin, 3: lutein, 4: zeaxanthin, 5: 5,6-epoxy-β-carotene, 6: α-carotene, 7: β-carotene, 8: 9Z – β-carotene, 9: 15Z – β-carotene**

Conclusions

The obtained data reveals the way in which the carotenoid pattern is affected by high light stress in the analyzed algal strain, as well as the way this reacts during the recovery stage.

They proved that the xanthophyll cycle's regulatory mechanism is functional in *Mougeotia sp*. algae, leading to an almost complete interconversion of violaxanthin to antheraxanthin and zeaxanthin. However, its contribution to non-photochemical quenching is not as significant as in higher plants; the small amounts of zeaxanthin recorded during experiments suggesting that this strain posses another dissipation mechanism(s) which operates together with xanthophyll cycle.

Hence, HPLC analysis revealed a particular behavior of *Mougeotia spp*. algae under intense illumination: the major de-epoxidation product of violaxanthin is not zeaxanthin, but antheraxanthin. More than that, the high light stress affects the whole carotenoid biosynthesis, starting with the violaxanthin cycle's precursor: β-carotene.

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P55 SIMULTANEOUS ION CHROMATOGRAPHIC DETERMINATION OF ANIONS AND CATIONS IN SURFACE WATERS FROM FIZES VALLEY

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Introduction

Water Framework Directive[11] demands a concerted approach in order to achieve a good ecological state for all water bodies across Europe. The objectives agreed must be coordinated beyond the level of individual survey areas and consolidated for the river basin district as a whole.

Fizes catchement, part of Somes catchement tributary of Tisa watershed, is located in Transylvania Plain, in the northern part of Romania. A distinctive feature of this area is the presence of ponds, mainly used for fishery. The major pollution sources are represented by sediments (generated by erosional processes and transported in the water bodies) and diffuse pollution sources (originated from agricultural activities and the improper septic systems of the localities). Fizes catchement, through its features of relatively low antropic pressures and with little structural changes, represents a natural laboratory for designing and implementing programs of restorations of watersheds in agricultural landscapes.

In such a context, chemical analysis is usually employed to identify the aquatic system characteristics including the assessment of inputs, distribution of various chemical species and characterization the outputs generated by the physical, chemical and biological processes developed within the water bodies. Among the specific chemical indicators, the inorganic species hold an important place, determining largely the behavior and evolution of the aquatic system.

The new analytical techniques, generated by the advent of ion chromatography (IC) deliver a more precise measurement of the various inorganic species present in the water body. IC is a high-performance ion-exchange chromatography technique for the separation and quantification of low-molecular-weight ions[1–8], being in use since 1975, from the time of the development of the eluent suppressor[6]. Because of its high accuracy and reliability, IC is nowadays the one of the most powerful tool for analysis of environmental samples[1,5], becoming an important technique for the determination of ionic species for monitoring water quality. This technique was used for the system of fishing ponds, streams and ground waters from Fizes Valley watershed (Fig. 1.), which was studied in order to assess the effects of antropic pollution through leaching of fertilizers from soils and waste waters from the villages within its catchement.

An IC method with conductivity detection was developed, enabling the simultaneous determination of six cations (Li^+, Na^+, K^+, NH_4^+, Mg^{2+} and Ca^{2+}) and seven anions ($F^–$, $Cl^–$, $Br^–$, $NO_2^–$, $NO_3^–$, $PO_4^{3–}$ and $SO_4^{2–}$) in a single run, saving thus analytical time, sample pre-treatment and reagents.

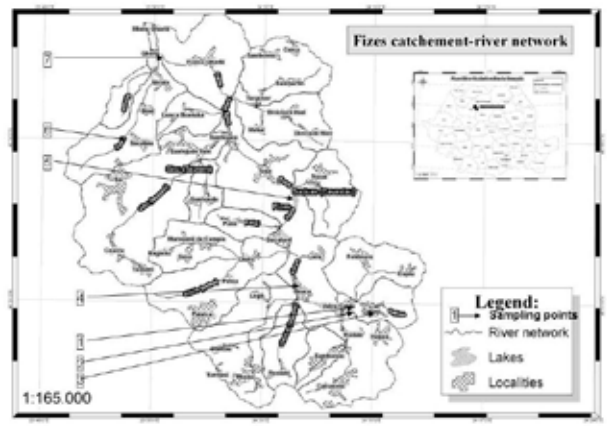

Fig. 1. **Fizes catchement river network map, with sampling points' locations**

Experimental

Chemicals for mobile phases' preparation were of analytical grade: 4-hidroxybenzoic acid (Acros Organics), lithium hydroxide (Scharlau) and nitric acid (Merck). Ultrapure water with a specific resistance of 18.2 $M\Omega\,cm^{-1}$ was utilized for preparation of mobile phases as well as for sample dilution, being obtained from a Direct Q 3UV Smart (Millipore). All solutions were stored in polyethylene bottles which had been thoroughly rinsed with ultrapure water. Mobile phases were filtered through a 0.45 μm membrane (Millipore), and then degassed using an Elmasonic S30 H ultrasonic bath before use. Standard working calibration solutions were prepared from a "six cation standard-II" (Dionex Corporation) and from "seven cation standard-II" (Dionex Corporation). The external standard method was used for quantification.

Water samples were collected from seven sources located in Fizes watershed; samples were passed through a 0.45 μm membrane filter (Millipore), and then were stored for 24 hours at 4 °C in 0.5 dm^3 polyethylene containers[9,10], each sample was analyzed in triplicates. The samples with ion concentrations exceeding the calibration range were diluted accordingly and re-analyzed.

Analyses were performed on a Shimadzu system, consisting from: a Proeminence DGU 20As online degasser, a Proeminence LC-20AP solvent delivery module, an automatic sample injector SIL-10AF, a conductivity detector CDD-10Avp, a Proeminence CTO-20A column oven, a FCV-10AH_2 valve unit, an Allsep Anion 7u column (150 × 4.6 mm), an Universal Cation 7u (100 × 4.6 mm) and a Proeminence CBM-20A system controller in a configuration which is represented schematically in Fig. 2. Instrument control, data acquisition and data analysis were accomplished by a computer running "LCsolution" ver.1.2. software.

300 μl samples were injected in each case; using a temperature of 40 °C, a total separation of 23 min. was effective for a good resolution for all seven anions from the mixed

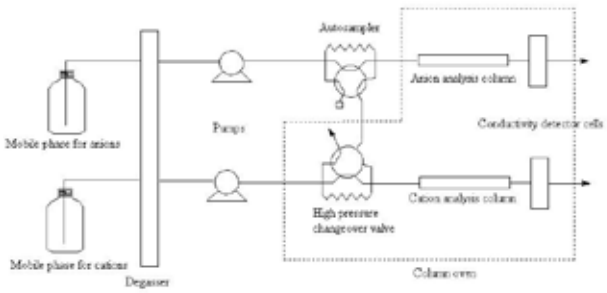

Fig. 2. **Schematic representation of the IC system configuration used for simultaneous analysis of cations and anions**

standard solution (fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate – Fig. 3.) and for all six cations (lithium, sodium, ammonium, potassium, magnesium and calcium – Fig. 4.), all peaks being baseline separated.

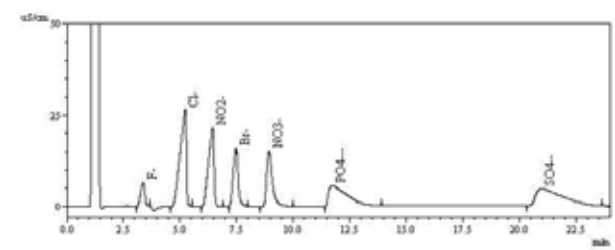

Fig. 3. **Separation for a standard mixture of anions (Allsep Anion 7u column, using as mobile phase a 4-hidroxybenzoic acid 4mM solution with pH-ul adjusted to 7.5 with LiOH 0.1M, the flow rate being 0.85 ml min^{-1})**

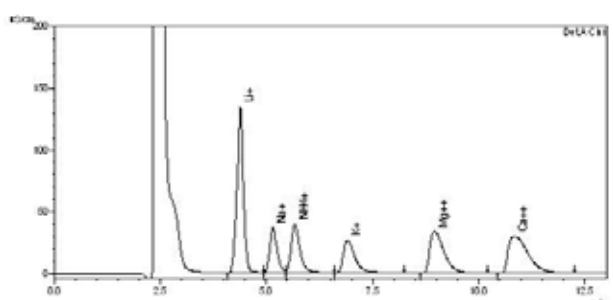

Fig. 4. **Separation for a standard mixture of cations (Universal Cation 7u column, using as mobile phase a 3 mM HNO3 solution, the flow rate being 0.5 ml min^{-1})**

A LGC certified reference material (LGC6020, SPS ww2) was used for validation.

Results

Calibrations were achieved using five levels of concentration, for accurately determine the concentration of target ions. The calibration curves show a good linearity with $R > 0.99$ as indicated in Table I, Figs. 5. and 6.

Table I
Results of regression analysis for calibrations

Anion	Linearity range [ppm]	Regression equation	R
F^-	4.02–20.10	C = 0.000130278A + 0.83973	0.9978
Cl^-	20.40–102.00	C = 0.000117938A – 0.523926	0.9997
NO^{2-}	20.20–101.00	C = 0.000156194A + 0.11461	0.9997
Br^-	20.00–100.00	C = 0.000230581A + 1.34467	0.9995
NO^{3-}	20.00–100.00	C = 0.000214872A + 0.28929	0.9999
PO_4^{3-}	40.00–200.00	C = 0.00023591A – 0.68846	0.9994
SO_4^{2-}	9.94–79.52	C = 0.0001677A – 1.1364	0.9994
Li^+	0.99–4.99	C = 0.00000262A – 0.7806	0.9993
Na^+	4.06–20.30	C = 0.000015644A – 2.12366	0.9997
NH_4^+	10.30–50.60	C = 0.000015822A + 1.04561	0.9999
K^+	5.02–25.10	C = 0.000000898A + 0.62187	0.9997
Ca^{2+}	5.04–25.20	C = 0.000000475A + 0.41618	0.9998
Mg^{2+}	10.18–50.90	C = 0.000011512A + 1.09553	0.9998

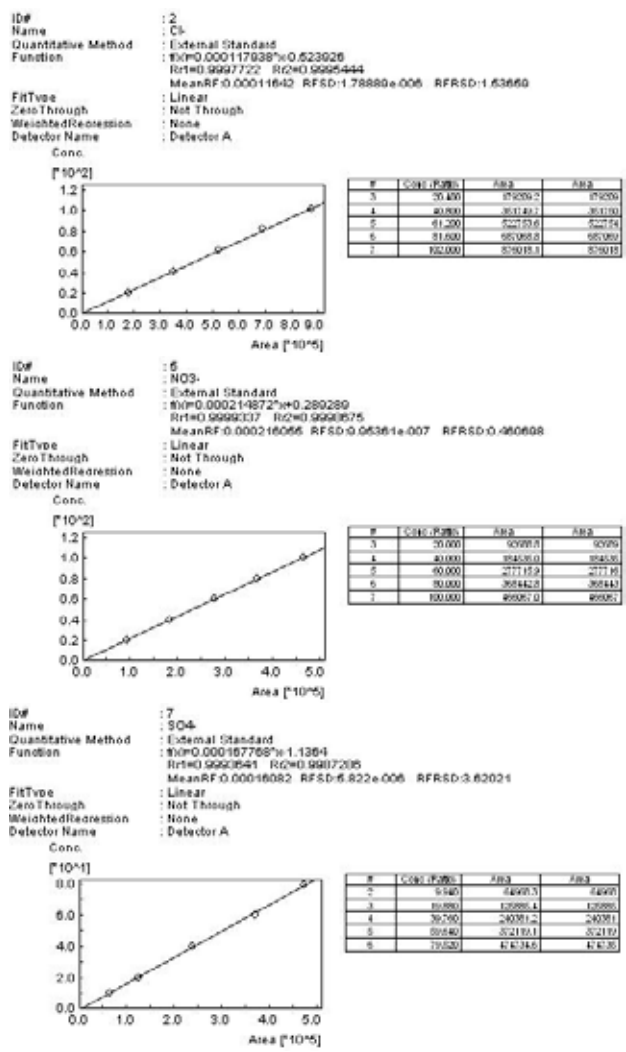

Fig. 5. **Calibrations for the reported anions**

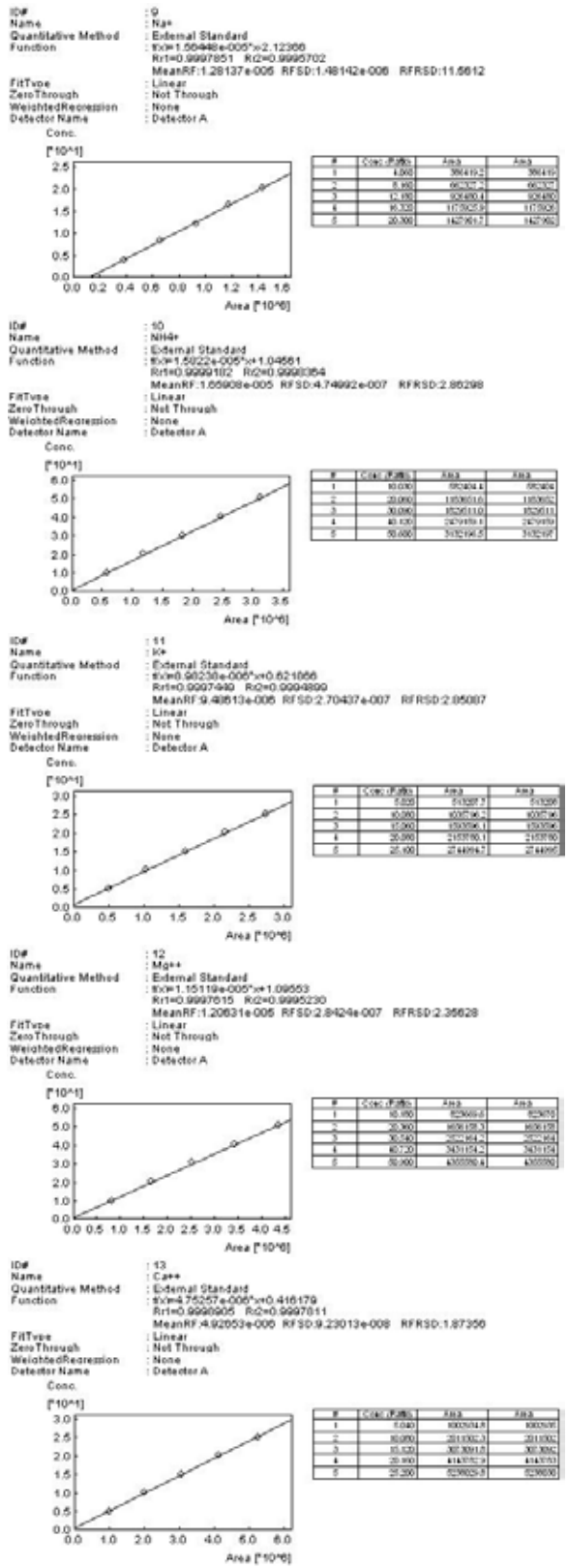

Fig. 6. **Calibrations for the reported cations**

From the 13 quantifiable ions, only eight were found in the analyzed water samples: Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Cl^-, NO_3^-, PO_4^{3-} and SO_4^{2-}, within a concentration range of 6.64 ppm (for K^+) to 346.27 ppm (for SO_4^{2-}). Calibrations for these ions are presented in figures 5 and 6.

Table II provides information on the anion concentration while Table III reveals the cation concentration from the investigated surface waters.

Table III
The anions' concentrations in the studied water sources (mean values ± SD)

Location	Cl^- [ppm]	NO_3^- [ppm]	SO_4^{2-} [ppm]
Draw well near Tăul Popii lake	66.99 ± 3.29	79.97 ± 3.97	343.28 ± 17.14
Spring near Tăul Popii lake	27.53 ± 1.39	–	141.17 ± 7.03
Cătina lake	66.54 ± 3.37	25.63 ± 1.21	346.27 ± 17.31
Geaca lake	57.21 ± 2.81	23.69 ± 1.15	264.03 ± 13.29
Ţaga lake	94.11 ± 4.70	23.02 ± 0.93	279.85 ± 13.85
Ştiucii lake	60.28 ± 2.96	–	63.69 ± 2.91
Fizeş river	191.53 ± 9.46	–	254.32 ± 12.62

The higher nitrate concentration in the draw well is due to the fact that a relatively high nitrate concentration is a general characteristic for underground water resources in Fizes catchement; this catchement has substantial diffusion pollution sources originated by manure and animal breeding. The chloride concentration ranges from 27.53 ppm (in a spring located near Tăul Popii lake) to 191.53 ppm (Fizes river), possible to explain due to the geological substrate of the area, salt being present as outcrops in the lower part of the catchement. The sulfate concentration was high in all samples, ranging from 63.69 ppm (in Stiucii lake) to 347.26 ppm (in Cătina lake).

All the concentration values are consistent with the general geological composition of the area. Slightly different values measured in different points of water surface sampling points in the same collector (Fizes valley) stream could be explained as consequence of normal variation due to different water contact duration among the watershed, during precipitation events.

Conclusions

This research revealed the state of the water quality and also clarified some aspects related to the process of self purifications of the water system in the considered area. The upstream ponds retain most of the sediment and pollutants through mechanisms of sedimentation and self-purification, most of the pollution sources being also located in the upper part of the catchement.

Data gathered will serve as beneficial experience for future rehabilitation measures. Using the proposed IC configuration, the laboratory productivity increases much, as there is no longer necessary to prepare two sample sets – one for

Table III
The cations' concentrations in the studied water sources (mean values ± SD)

Location	Na^+ [ppm]	NH_4^- [ppm]	K^+ [ppm]	Mg^{2+} [ppm]	Ca^{2+} [ppm]
Draw well near Tăul Popii lake	121.60 ± 6.03	15.28 ± 0.73	20.96 ± 1.03	108.05 ± 5.33	76.17 ± 3.80
Spring near Tăul Popii lake	25.93 ± 1.26	12.02 ± 0.51	6.64 ± 0.30	69.27 ± 3.42	65.43 ± 3.25
Cătina lake	168.31 ± 8.41	12.31 ± 0.44	15.54 ± 0.72	156.96 ± 7.79	76.27 ± 3.73
Geaca lake	158.05 ± 7.84	14.62 ± 0.70	17.52 ± 0.86	151.21 ± 7.53	71.25 ± 3.48
Ţaga lake	103.47 ± 5.15	13.03 ± 0.63	19.15 ± 0.94	104.22 ± 5.19	54.07 ± 2.65
Ştiucii lake	28.79 ± 1.40	12.65 ± 0.59	18.43 ± 0.90	18.80 ± 0.93	9.10 ± 0.41
Fizeş river	275.94 ± 13.78	11.11 ± 0.36	15.52 ± 0.74	142.90 ± 7.17	74.97 ± 3.74

anion analysis, the other for cation analysis. With one injection, the autosampler introduces the sample in both analysis channels.The method has potential to be used in water quality surveys in the purpose of correlating diffuse pollution inputs with changes in water quality. Specific self purification processes can also be operatively estimated.

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P56 STRATEGIES TO REDUCE DETECTION LIMITS IN THE ANALYSIS OF BROMINATED FLAME RETARDANTS IN ENVIRONMENTAL SAMPLES

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Introduction

Polybrominated diphenyl ethers (PBDEs) represent an important class of brominated flame retardants (BFRs) which are widely used in various consumer products such as electronic equipment, textiles and plastics[1]. These chemicals are highly persistent and bioaccumulative what leads to their ubiquitous occurrence in the environment[2], both in abiotic and biotic matrices.

Compared to major group of organohalogenated persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), the levels of PBDEs in respective environmental compartments are typically lower by one order of magnitude. On this account, low detection limits (LODs) are needed for their reliable control.

Gas chromatography coupled to mass spectrometry (GC/MS) operated in either electron ionization mode (EI) or in negative chemical ionization (NCI) are commonly employed analytical procedures of determination these compounds[3]. In NCI mode monitoring of abundant bromine ions (m/z 79 and 81) provides a more sensitive and selective option compared to EI. Nowadays, comprehensive two-dimensional (orthogonal) gas chromatography coupled to time-of-flight mass spectrometry (GC × GC/TOFMS) has become another challenging alternative to analyze very complex PBDEs mixtures[4].

In any case, achieving low LODs is also associated with the amount of sample introduced into GC system. The most commonly used GC injection technique for PBDEs is a splitless injection mode[3], however, several studies were reported a possibility to employ a large-volume programmed-temperature vaporizer injection (PTV) in the determination of these compounds[5].

This paper presents the method performance characteristics obtained in several GC systems used for quantification of PBDEs.

Experimental

For our experiments a standard mixture of most common PBDE congeners (BDEs No. 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183) purchased from AccuStandard inc. (USA) was used. The real-life sample containing trace amount of PBDEs was a purified extract obtained from fish tissue by procedure described by Hajšlová et al.[6]. To assess LODs achievable under various GC conditions following set-up were tested:

- PTV-GC/MS (EI),
- PTV-GC/MS (NCI),
- GC/TOFMS (EI),
- PTV-GC/TOFMS (EI).

PTV-GC/MS (EI) and PTV-GC/MS (NCI)

GC/MS analyses were performed on an Agilent 6890N gas chromatograph coupled to a mass selective detector (Agilent 5975XL Inert MSD) equipped with quadrupole analyzer operated in NCI or EI mode using splitless or PTV injection. The GC conditions were as follows: a DB-XLB capillary column (15 m × 250 μm i.d. × 0.1 μm, J&W Scientific); a oven temperature program: from 105 °C (held for vent time) to 260 °C (held for 1 min) at 50 °C min^{-1} then to 300 °C at 20 °C min^{-1} and held for 3 min; carrier gas: helium with constant flow 1.5 ml min^{-1}. The MS was operating in the selected ion monitoring (SIM) mode (monitored ions were m/z 79, 81, 159, 161 and m/z 406, 484, 564, 484, 562 for NCI and EI mode, respectively). The MS (NCI) parameters were as follows: reagent gas: methane; temperatures of MSD interface, ion source, and quadrupole: 280 °C, 150 °C, and 150 °C, respectively. The temperatures of MSD interface, ion source and, quadrupole for MS (EI) system were 280 °C, 230 °C and, 150 °C, respectively.

Four parameters for PTV injection were tested: vent time (*VT*), vent flow (*VF*), injection volume (*IV*) and splitless period. Starting injection temperature was 50 °C (held 4.6 min) and it was ramped to 350 °C at 500 °C min^{-1}.

GC/TOF MS and PTV-GC/TOF MS

The analyses were performed on a Pegasus 4D instrument (Leco, USA) consisting of an Agilent 6890N gas chromatograph equipped with splitless and/or PTV injector and a Leco Pegasus III high-speed time-of-flight mass spectrometer.

The same, DB-XLB capillary column was used for determination of analytes. The GC conditions were similar to PTV–GC/MS system. The interface temperature was 280 °C. The MS acquisition rate was 11 Hz, the mass range 35–850 amu, the ion-source temperature 300 °C, and the detector potential –1875 V.

Results

In the first part of this study, the implementation and optimalization of a PTV injection coupled with GC/MS (EI and/or NCI mode) was realized. A solvent standard solution of above mentioned PBDE congeners was used for optimalization PTV injection conditions. Optimal parameters assessed by the comparison of a peak height of individual analytes were as follows:

- vent time: 90 s
- vent flow: 60 ml min^{-1}

Table I
LODs of individual PBDE congeners [ng ml^{-1} isooctane] for the GC/MS techniques tested

Analyte	GC/MS (EI) (splitless)	GC/MS (NCI) (splitless)	PTV–GC/MS (EI)	PTV–GC/MS (NCI)	GC/TOFMS (EI)	PTV–GC/TOFMS
BDE 28	1.0	0.05	0.1	0.01	2.3	0.3
BDE 47	1.0	0.1	0.1	0.01	3.1	0.6
BDE 49	1.0	0.1	0.1	0.01	2.5	0.4
BDE 66	1.0	0.1	0.1	0.01	3.7	0.6
BDE 85	2.0	0.2	0.3	0.01	1.3	0.7
BDE 99	1.5	0.1	0.3	0.01	3.0	0.4
BDE 100	1.5	0.1	0.1	0.01	0.6	0.6
BDE 153	5.0	0.1	0.6	0.02	3.2	0.9
BDE 154	3.5	0.1	0.3	0.02	2.5	0.7
BDE 183	3.0	0.2	0.2	0.06	7.1	1.5

- injection volume: multiple injection 2 × 10 µl
- splitless period: 2 min.

The repeatability of the PTV–GC/MS injection, expressed as a relative standard deviation (RSD, n = 10) ranged from 3.8 to 6.3 % and 3.8 to 10.3 % for NCI and EI mode, respectively. Similar RSD values for multiple injections were obtained (3.8–9.2 % and 1.7–10.8 % for NCI and EI mode, resp.) were determined for spiked fish lipid extract (1 ng g^{-1} lipid weight).

LODs calculated as a quantity of analyte that generates a response 3-time higher than the noise level of the detection system (based on the injection of solvent standard solution mixture) are summarized in Table I. LOQs were the minimum concentrations of analytes that was possible to quantify with acceptable accuracy and precision. Under these conditions, the LOQ was the lowest calibration level and corresponded for particular analyte to 3 × LOD. Generally, in NCI mode, significantly lower LODs were obtained compared to EI mode. On the other hand, the identification of individual PBDEs was only based on their retention times. PBDEs are eluted in order of increasing bromine number and intensity of appropriate responses was obtained in following order in case of GC/MS (NCI) system: BDE 28 > BDE 49 ~ BDE 47 ~ BDE 66 ~ BDE 100 > BDE 99 ~ BDE 85 ~ BDE 154 ~ BDE 153 > BDE 183 (see Fig. 1.). Similar chromatogram obtained by PTV-GC/TOFMS (EI) is documented in Fig. 2.

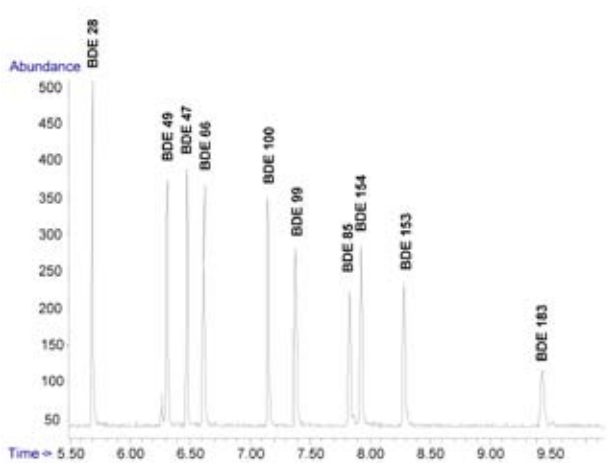

Fig. 1. **Chromatogram of mixed standard of PBDEs obtained by GC/MS (NCI)**

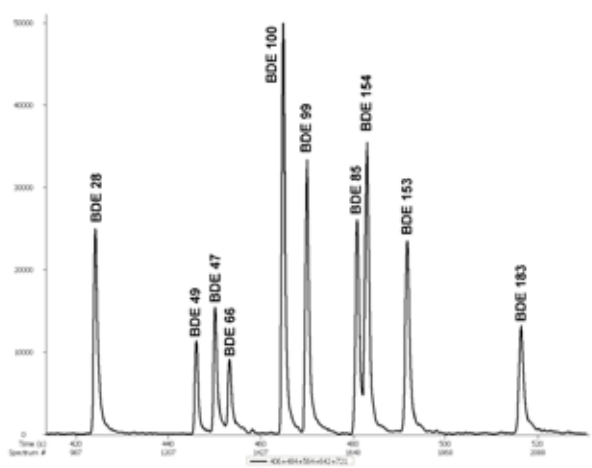

Fig. 2. **Chromatogram of mixed standard of PBDEs obtained by PTV–GC/TOFMS (EI)**

Table I shows a significant reduction of LODs by almost one order of magnitude (to values 0.01–0.06 ng ml^{-1} by the use of PTV which allowed introduction of 1–250 µl of sample (only 1–5 µl were injected by pulsed splitless).

PTV–GC/TOFMS provide LODs similar to PTV–GC/MS (EI) and values were ranged from 0.3 to 1.5 ng ml^{-1}. The main advantage of this technique is availability of full mass spectral information for all sample components, i.e. confirmation of target analytes.

Conclusions

In the present work, LODs of individual PBDE congeners were reduced using various GC systems in analysis of these analytes in various matrices such as environmental samples (fish muscle, sediment etc.). Four different injection

techniques, three of them employing PTV, were tested in our study.

- Generaly, the NCI mode provided signifficantyl lower LODs compared with EI mode coupled to all tested GC/MS system.
- The lowest LODs were obtained by GC/MS coupled to the PTV injection operated in NCI mode, under these conditions large quantity of analytes without solvent (due to PTV injection) and high selectivity for bromine ions (due to MS-NCI mode) could be performed.
- PTV-GC/TOFMS employing splitless injection provided similar results in comparison with GC/MS (NCI) using splitless injection.

This study was undertaken within the projects MSM 6046137305 and NPV II (2B06151) both supported by the Ministry of Education, Youth and Sports of the Czech Republic.

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P58 SIMULTANEOUS DETERMINATION OF MERCURY, LEAD AND CADMIUM IN AQUEOUS SAMPLES USING PECTIC ACID-MODIFIED CARBON PASTE ELECTRODE

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Introduction

Anodic stripping voltammetry (ASV) and its variants are the most sensitive electroanalytical techniques employed for trace heavy metal analysis. Mercury electrodes have been widely used for ASV due to their ease of use and compatibility with a number of heavy metal species. Recently, however, there are major concerns regarding the use of mercury in the laboratory because of hazards it poses to humans and other living organisms. Prompted by environmental issues, some of the applications of modified carbon paste electrodes (CPE) are aimed at the development of mercury-free electrodes for ASV[1]. In this paper, we investigated the use of pectic acid as a modifier for CPE and its utilization for the analysis of some representative heavy metal species.

Pectic acid, also known as polygalacturonic acid, is a natural polymer found in citrus rinds. It consists of chains of 300 to 1000 units of galacturonic acid monomer joined with $\alpha\,1 \rightarrow 4$ linkages. When incorporated in a CPE, the exposed carboxylic acid groups are responsible for the accumulation of heavy metal ions on the electrode surface presumably through ion-exchange or complex-formation processes.

The aim of this study is to fabricate pectic acid-modified CPE and examine various experimental conditions that affect the analytical signal when used as a working electrode for the ASV of mercury, lead and cadmium in aqueous samples.

Experimental

Pectic acid isolated from orange peel was obtained from Fluka and used without further purification. Mineral oil, graphite powder were purchased from Sigma-Aldrich while 1,000-ppm standard solutions of Hg (II), Pb (II) and Cd (II) were from Sharlau (Spain). All other chemicals used were at least AR grade.

All electrochemical experiments were carried out using a BAS 100B Electrochemical System (BioAnalytical System) in the Osteryoung square wave stripping voltammetry mode (OSWSV) or cyclic voltammetry (CV) utilizing Ag/AgCl reference electrode and a Pt wire counter electrode.

Modified carbon paste was prepared by thoroughly mixing 4 : 1 (w/w) ratio of graphite powder to powdered pectic acid and enough mineral oil to form a paste typical for conventional CPEs. A portion of prepared paste was then tightly packed on the cavity (2-mm dia.) of previously cut 200-ml pipette tip where a copper rod was inserted on the other end to establish electrical contact.

The modified CPE was first immersed in a sample solution containing the heavy metal ion being analyzed. After a predetermined period of time, referred to as accumulation time, the electrode was removed from the sample and rinsed thoroughly with water. The electrode was then transferred into a voltammetric cell containing deoxygenated 0.1M HCl for ASV. The accumulated metals were first reduced by applying a sufficiently negative potential of –800 mV for 60 s, then re-oxidized while anodically scanning the potential. The peak-type *I-E* plots resulting from the anodic scan were recorded and evaluated.

Results and Discussion

Preliminary Studies

Cyclic voltammetric experiments revealed that pectic acid-modified CPE possesses a useful potential window ranging from –900 mV to 1200 mV relative to the Ag/AgCl reference electrode when the supporting electrolyte used was deoxygenated 0.1M HCl. Preliminary investigations also showed that Cd, Pb and Hg undergoes redox transformations at about –690, –470 and +100 mV, respectively, using the above CPE and electrolyte combination. In principle, these metal species may be simultaneously detected and conveniently analyzed with well resolved analytical peaks using the modified CPE.

Individual Analysis

Results of ASV experiments using laboratory prepared solutions containing a single metal species are consistent with the literature[2,3]. For all the three heavy metal species, as the concentration increases or the accumulation time is prolonged, the peak current also increases in a linear fashion until such an instance where current signal plateaus and further increase in concentration or accumulation time does not anymore amplify the peak height. This is due to the fact that the higher the concentration or the longer the electrode is immersed into the solution containing the analyte; more metal ions are able to accumulate on the surface, which consequently provides a higher current. Saturation point is attained when the active sites for metal accumulation are maximized resulting to levelling of current response. Using an accumulation time of 2 min and other parameters described in the experimental section, the sensitivities and detection limits are reported in Table I. Sensitivities were calculated from the slopes of individual calibration curves within the linear dynamic range while detection limits (LOD) were estimated based on three times the standard deviation of the blank. Further

Table I
Analytical data for pectic acid-modified CPE

Metal	Sensitivity [μA ppm^{-1}]	LOD [ppm]	LOL [ppm]
cadmium	20	0.25	20
lead	50	0.15	50
mercury	10	0.40	50

improvements in LOD and limits of linearity (LOL) were observed upon increasing or decreasing the accumulation time, respectively.

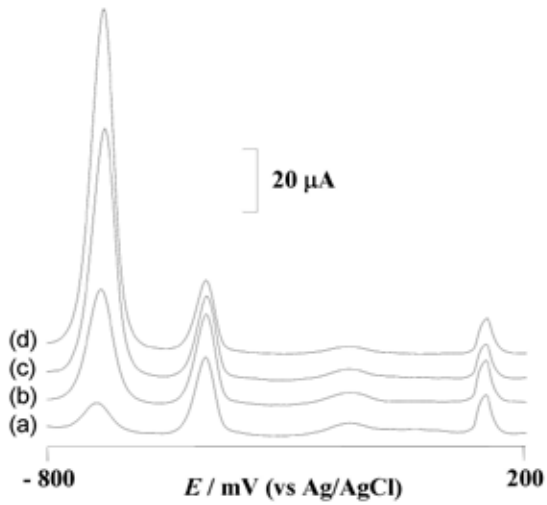

Fig. 1. **ASV for solutions containing 1 ppm Pb, 3 ppm Hg and varying Cd: (a) 1, (b) 3, (c) 5, (d) 10 ppm**

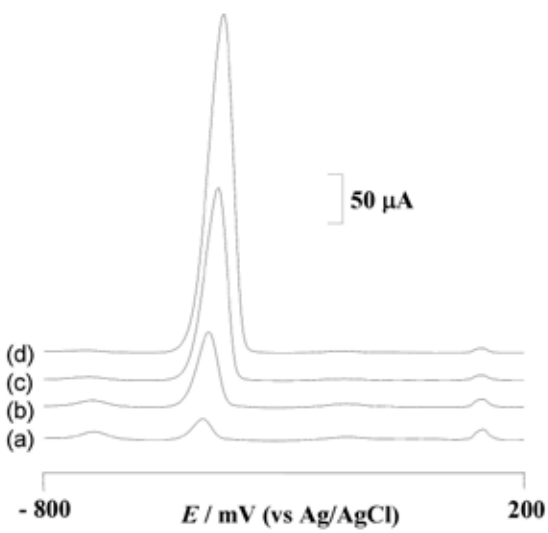

Fig. 2. **ASV for solutions containing 1 ppm Cd, 3 ppm Hg and varying Pb: (a) 1, (b) 3, (c) 5, (d) 10 ppm**

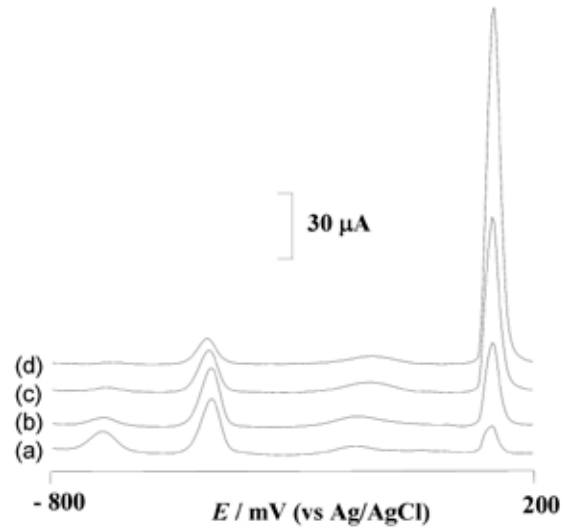

Fig. 3. **ASV for solutions containing 1 ppm Cd, 1 ppm Pb and varying Hg: (a) 3, (b) 5, (c) 10, (d) 20 ppm**

S i m u l t a n e o u s A n a l y s i s

When two or three metals studied in this project are present in the same solution, well resolved peaks with characteristic potentials corresponding to specific metal species are observed as shown in Figs. 1., 2. and 3.

Although linearity between peak height and concentration is maintained, careful inspection of individual peaks revealed that sensitivity decreases when the three metal ions co-exist. Moreover, as the concentration of one metal increases, the current signal for the other two decreases. This is presumably due to competition for binding sites among the metal ions and their varying affinities to carboxylate groups in pectic acid.

Acknowledgement (This work has been supported by the Ministry of Development of Brunei Darussalam).

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P59 DETECTION OF FOREIGN ORGANIC SUBSTANCES IN WATER AND BIOLOGICAL SAMPLES

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Introduction

The chemical analysis of biomedical samples (bodily fluids, tissues) aimed at revealing exposure to toxic chemicals (TCs) can be directed to the following targets:

(i) TCs themselves, when their metabolism is slow enough,

(ii) Low-molecular metabolites of TCs,

(iii) High-molecular adducts of TCs with proteins.

Right choice of target (marker) with account for the life cycle of a TC (adsorption–distribution – metabolism – excretion) predetermines success of analysis. The targets *(i)* and *(ii)* are more convenient to determine by conventional GC-MS methods but are unsuitable for retrospective analysis in view of their short life time in the organism. We developed procedures for the determination in biomedical samples of all the three groups of biomarkers of TCs.

Results and Discussion

Direct analysis of a TC in biomedical samples was considered to be a rational approach in toxicokinetic research on fluoroacetic acid, one of the most potent metabolic poinsons (FAA). Salts of FAA are still used in some countries for rodent population control; deadly poisoning of humans and farm animals was also described. At our laboratory, procedures for the determination of O-alkyl esters of methylphosphonic acid (low-molecular metabolites of organophosphorus warfare agents, OPWAs) and thiodiglycol (metabolite of sulfur mustard) were also developed. These metabolites are products of both biogenic and abiogenic hydrolysis of the parent agents, and, therefore, their determination in environmental samples is actual for retrospective analysis aimed at establishing the fact and degree of environmental pollution with the corresponding agents. Of particular importance for forensic analysis are universal procedures suitable both for water and for biological fluids. We developed universal procedures for the determination of FAA, methylphosphonic acid (MPA), ethyl MPA (marker of VX), isopropyl MPA (marker of sarin), isobutyl MPA (marker of Russian VX), pinacolyl MPA (soman marker), and thiodiglycol in water, urine, and blood plasma.

For retrospective establishment of exposure to TCs, procedures for the determination of reactivation products of blood plasma butyryl cholinesterase (BChE) inhibited by OPWAs and of thiodiglycol isolated by alkaline hydrolysis from albumin adducts. These procedures all are based on GC/MS combined with solid-phase microextraction (SPME).

Solid-phase microextraction is a unique method that allows one to combine on a single stage extraction from a matrix, concentration, and injection of a sample. SPME offers a great advantage of analyzing the whole sample rather than its aliquot, and, therefore, is more than about an order of magnitude more sensitive compared with traditional separation and concentration methods. SPME poses no threat of contamination of a GCMS system by matrix components. Of key importance for successful SPME analysis is right choice of microfiber, conditions for sorption (temperature, time, sample mixing mode, ionic strength of analyzed solution) and desorption (temperature, delay time).

Therefore, in developing an SPME procedure, one should optimize the following parameters:

- Type of microfiber
- Sorption and thermodesorption temperatures of target analytes
- Sorption and thermodesorption times
- Ionic strength of the solution.

Before GC/MS analysis nonvolatile target compounds were derivatized either in situ with subsequent concentration of the volatile derivative on microfiber (analysis for FAA) or directly on microfiber with vapors of derivatizing agents (analysis for MPA and its O-alkyl esters).

In what follows we schematically represent certain of the mentioned procedures. Fig. 1. shows the block scheme of the determination of sodium fluoroacetate in various media. In view of the fact that the volatile derivative, ethyl fluoroacetate, is sampled from equilibrium vapor, the sample matrix scarcely affects the results of analysis. The developed procedure is universal and can be applied both for control of drinking water and for toxicokinetic and forensic investigations.

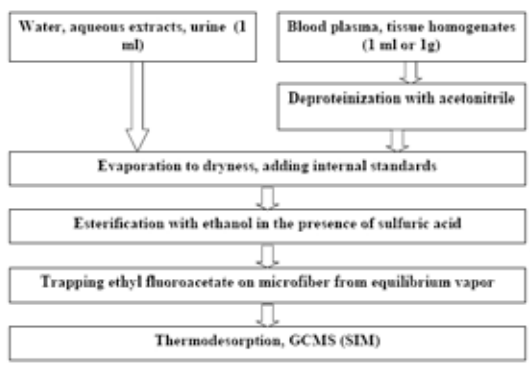

Fig. 1. **Block scheme of the unified procedure for the determination of sodium fluoroacetate in water and biomedical samples by SPME-GCMS**

The detection limits are 0.001 mg ml^{-1} for drinking and natural waters, 0.01 mg ml^{-1} for blood plasma, and 0.01 mg ml^{-1} for organ homogenates (without recounting for dry weigh). The procedure is described in detail in ref.[1].

For the determination of the low-molecular metabolites of organophosphorus warfare agents, MPA and its alkyl

esters, in urine we suggested a procedure involving extraction of the analytes on microfiber, their silylation with *N*-tert-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) directly on microfiber, thermodesorption of the resulting derivatives in a hot GC injector, GC separation, and MS detection in the SIM mode. Three types of microfibers of various polarities were tested: 50/30 μm DVB/Carboxen/PDMS, 85 μm Carboxen/PDMS, and 70 μm Carbowax/DVB (Fig. 1.). The best results were obtained on the first microfiber.

Unlike certain related techniques, SPME provides a unique possibility for experimenting with various types of microfibers differing from each other in chemical nature and micropore size. Development of an SPME procedure always begins with searching for an optimal microfiber. This process is illustrated in Fig. 2.

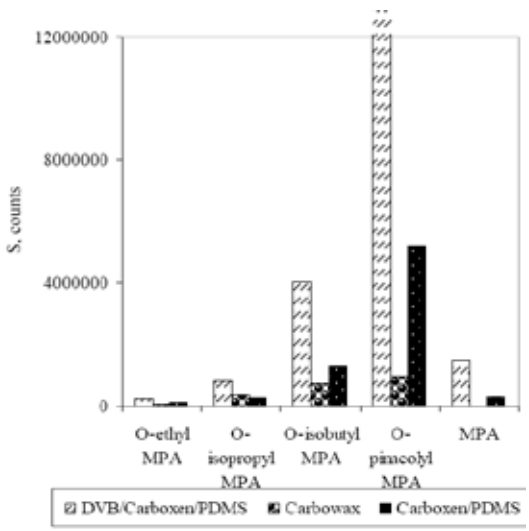

Fig. 2. **Efficiency of various microfibers for the determination of MPA and O-AMPAs as tert-butyldimethylsilyl derivatives**

In vivo animal (rats) experiments gave evidence for the possibility of revealing exposure to OPWAs at the ≥0.5 LD_{50} level within no less than 48 h after exposure. Urinary metabolites of OPWAs could, in principle, be detected within two weeks after exposure, but even if sufficiently high doses of OPWAs were applied. The procedure is schematically represented in Fig. 3 and described in detail in ref.[2].

For retrospective establishment of exposure to OPWAs we developed an SPME-GCMS procedure based on reactivation of inhibited BChE (Fig. 4.). Reactivation of blood BChE inhibited by OPWAs by the action of fluoride ion gives rise to the parent compounds in the case of G-type agents or fluoroanhydrides in te case of V-type agents. SPME is especially efficient in this case, since the reactivation products are trapped by microfiber and thus eliminated from the reaction zone, which drives the reactivation process. Soman is best retained by microfiber. In should be noted that the developed procedure is feasible for the determination of total soman and for the separate determination of reactivated and intact soman.

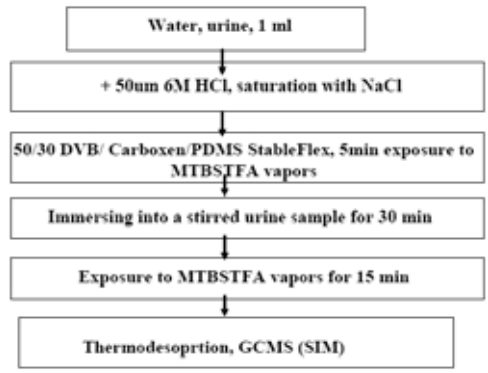

Fig. 3. **Block scheme of the procedure for the determination of O-AMPAs in urine by SPME-GCMS**

Conclusions

Procedures for the determination in biomedical samples of biomarkers of TCs have been developed. For fluoroacetic acid, the detection limits are 0.001 mg ml^{-1} for drinking and natural waters, 0.01 mg ml^{-1} for blood plasma, and 0.01 mg g^{-1} for organ homogenates (without recounting for dry

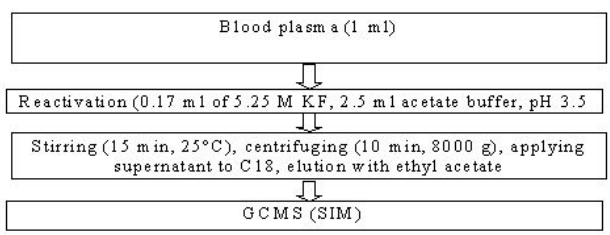

Fig. 4. **Block scheme of the determination of blood plasma BChE reactivation products by SPME-GCMS**

weigh). For organophosphorus warfare agents, the detection limits in the analyzed sample volumes are 0.01 mg dm^{-3} for sarin and 0.002 mg dm^{-3} for soman. The analysis time (including sample preparation) is 2.5 h. Exposure to OPWAs by the results of analysis for inhibited BChE reactivation products can be revealed within 2 and more weeks after intoxication with high doses.

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P60 DETERMINATION OF SULFONAMIDES IN WATER USING MULTI-WALLED CARBON NANOTUBES SPE AND HPLC WITH FLUORESCENCE DETECTOR

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Introduction

Multi-walled carbon nanotubes (MWCNTs) are a novel carbon material, repeatedely discussed in the literature for the solid phase extraction (SPE) of several organic contaminants.[1–3]

In this paper a sensitive method was developed for the determination of six commonly sulfonamides (SAs) in water by SPE using MWCNTs as adsorbent. Final analysis was carried out by HPLC coupled with fluorescence detection.

Experimental

Reagent and Water Samples

Sulfadimethoxine (SDM), sulfadiazine (SDZ), sulfamethoxazole (SMX), sulfamerazine (SMR), sulfanilamide (SNA), sulfaguanidine (SGN) and fluorescamine were obtained from Sigma-Aldrich. MWCNTs were purchased from Institute for Research and Development of Isotopic and Molecular Technologies Cluj-Napoca, Romania. Deionized and redistilled water was prepared on Milli-Q Plus (Millipore). Acetonitrile, ortophosphoric acid (H_3PO_4) and dipotassiumphosphate (K_2HPO_4) were purchased from Merck. A river water sample was collected from Somes (Cluj-Napoca) and filtered through 0.45 μm nylon membrane and stored at a temperature of 4 °C.

Chromatographic System and Conditions

All experiments were carried out by using Shimadzu VP Series liquid chromatograph equipped with a degasser and a mixer of mobile phase. A fluorescence (FL) detector FR-10 AXL with excitation wavelength of 405 nm and emission wavelength of 495 nm was used to analyse the tested solutions. Chromatographic separation was performed on a Alltima RP C-18 column (250 mm × 4.6 mm, 5 μm). Gradient elution with a mixture of acetonitrile (solvent A) – phosphate buffer pH 3.5 (solvent B) at a flow rate of 1 ml min^{-1} was applied. The initial gradient conditions were: 65 % solvent B for the first 25 min, decreasing to 50 % in 25 min, finally it was brought back to 65 % in 5 min and held for 5 min until the next injection.

Extraction Procedure

The cartridge packed with 200 mg MWCNTs was prepared in a 6ml polypropylene syringe and the sorbent was retained by two polyethylene frits. The solutions (water sample or water sample spiked with analytes) adjusted to pH 6 were loaded at a flow rate of 4 ml min^{-1}.Then, SAs were eluted using a mixture of 3 ml ammonium acetate water solution (0.2M) and 6 ml acetonitrile.The eluate was evaporated to about 3 ml under nitrogen stream in a 35 °C block heater. Then 3 ml of methylene chloride was added and each samples was mixed and separated. The lower layer was evaporated to near dryness. The extract was reconstituted in 1.0 ml mobile phase. The analytes were quantified by HPLC with a pre-column derivatization with fluorescamine (400 μl of sample + 400 μl of fluorescamine 0.1 %).The whole solution was mixed with a vortex mixer. The sample was filtered through a 0.45 μm nylon filter and after standing for 30 min at ambient temperature was ready for analysis.

Results

The effect of the pH was investigated over the range of pH 4–8 and it was found that the pH of sample solutions in the whole range nearly had no influence on the extraction of SAs.

To investigate the influence of sample volume, different volumes of pure water were spiked with a constant mass of 0.25 μg of each analyte. It was found that the recoveries of SAs
decreased slightly with the increase of sample volume. When the volume was 200 ml, the recoveries of 55–93 % were obtained for the six SAs.

For elution of SAs we selected a mixture of ammonium acetate and acetonitrile 1 : 2 (v/v) and better recoveries were obtained when eluent volume amounted to 9 ml.

The results of the linearity of SAs determined under the optimized conditions and using 200 ml of spiked pure water are reported in Table I. The linearity of each compound measured by HPLC method was good from 0.05 to 5 ng ml^{-1}.

The correlation coefficient of the calibration curves were above 0.999.

Table I
Retention time (t_R) and linearity of SAs

Sulfonamide	t_R [min]	b[a]	a[b]
SMX	11.23	21.694	0.564
SNA	17.69	20.944	0.574
SDZ	20.43	9.402	0.245
SMR	22.94	16.644	–0.192
SGN	42.32	8.021	0.071
SDM	46.68	5.560	0.104

b[a]: Slope
a[b]: Intercept

The recoveries of analytes were evaluated using 200 ml of environmental water samples (Somes river) spiked with

the mixture of SAs at 0.25 and 2.5 ng ml^{-1} and enriched and analyzed by this system. The results are listed in Table II.

Table II
Recoveries of SAs spiked water samples (n = 3)

Sulfonamides	Spiked [ng ml^{-1}]	Recovery [%]
SMX	0.25	57 ± 5.2
	2.5	55 ± 6.4
SNA	0.25	74 ± 7.3
	2.5	73 ± 6.8
SDZ	0.25	82 ± 4.3
	2.5	77 ± 3.7
SMR	0.25	58 ± 5.4
	2.5	56 ± 7.6
SGN	0.25	93 ± 4.1
	2.5	88 ± 6.9
SDM	0.25	87 ± 2.5
	2.5	79 ± 5.3

The chromatogram of the river water sample spiked with SAs is shown in Fig. 1.

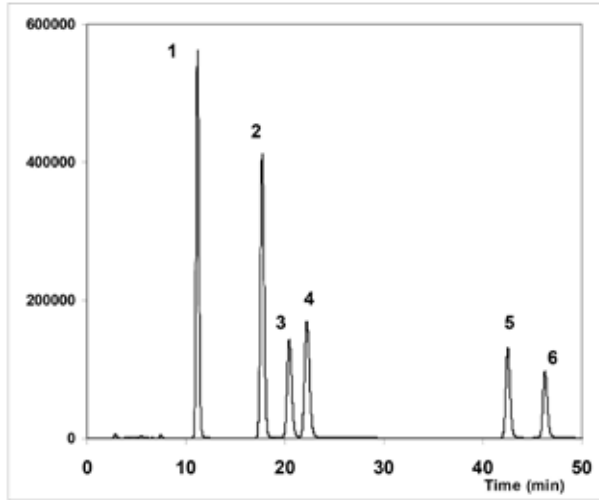

Fig. 1. **SPE-LC-FL chromatogram of river water sample spiked with 2.5 ng ml^{-1} of each compound. (1) SMX; (2) SNA; (3) SDZ; (4) SMR; (5) SGN; (6)SDM**

Conclusions

The HPLC method with fluorescence detection characterized by a good reliability for quantitative determination of six different kinds of SAs from environmental samples has been developed. The results showed that MWCNTs could be used as a potent SPE sorbent for SAs.

This work has been supported by the Natio-nal Authority for Scientific Research of Romania, CEEX project no. 62/2006.

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P61 MICROBIOLOGIAL REMEDIATION OF METAL-CONTAMINATED SOIL

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Introduction

Soil contamination with anthropogenic heavy metals, which mainly comes from industrial activity, atmospheric deposition and land application of sewage sludge, has received much attention in the recent years. The anthropogenic heavy metals are to be easily accumulated in the topsoil1, resulting in potential problems such as toxicity to plants and animals[1,2], accumulation in food chain, perturbation of the ecosystem and adverse health effects[4,5]. Metals, which are significantly toxic to human beings and ecological environment, include chromium (Cr), cooper (Cu), lead (Pb), mercury (Hg), zinc (Zn), manganese (Mn), cadmium (Cd), nickel (Ni), arsenic (As) and iron (Fe), etc.[6].

Contaminated soil is notoriously difficult to treat because the contaminants are often tightly bound to the soil particles. Conventional remediation technologies are becoming less popular due to the high treatment costs and bioremediation processes to improve the contaminant removal efficiency and cost effectiveness. However, as an innovative technology, there are many factors to be investigated with the future development[7].

Bioremediation

Bioremediation is defined as a method using living organisms, or their particles (enzymes) to reduce, eliminate, fixate or transform contaminants presented in the soil, in sediments, in waters or in the air[8,9]. In the bioremediation processes bacteria, fungi, yeasts and plants present the most imposed organisms. Recently, the ability of algaes and planktons are being researched. Some technologies are based on the use of general or genetically modificated organisms[8]. Bioremediation works by either transforming or degrading contaminants to non-hazardous or less hazardous chemicals. These processes are called, respectively, biotransformation and biodegradation. Biotransformation is any alteration of the molecular or atomic structure of a compound by microorganisms. Biodegradation is the breaking down of organic substance by microorganisms into smaller organic or inorganic components[9].

Metal – Microbe Interactions

Microorganisms, especially bacteria, exist in complex biogeochemical matrices in subsurface sediments and soils[9] and are known to mediate many geochemical processes[10,11]. They can interact with metals via many mechanisms, some of which may be used as the basis of the potential bioremediation strategies[9]. Microbes can mobilize metals through autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, and methylation, which can result in volatilization. Conversely, immobilization can result from sorption to cell components or exopolymers, transport into cells and intracellular sequestration or precipitation as insoluble organic and inorganic compounds, e.g. oxalates[12,13] sulphides or phosphates[14,15].

Metal Mobilization

Bioleaching. Metals can be leached from solid matrices via autotrophic and heterotrophic leaching. Chemolitotrophic and heterotrophic bacteria have the major role.

Most autotrophic leaching is carried out by chemolithotrophic, acidophilic bacteria which fix carbon dioxide and obtain energy from the oxidation of ferrous iron or reduced sulfur compounds, which causes the solubilization of metals because of the resulting production of Fe(III) and H_2SO_4[16,17]. The microorganisms involved include sulfur-oxidizing bacteria, e.g., *Acidihiobacillus thiooxidans*, iron- and sulfur-oxidizing bacteria, e.g., *Acidithiobacillus ferrooxidans* and iron-oxidizing bacteria, e.g., *Leptospirillum ferrooxidans*[18,19].

In the case of oxide, carbonate and silicate ores, limits are set for the use of thiobacilli. For such ores, research is being done on the use of heterotrophic bacteria. In this case, metals are dissolved by organic acids, or complexing, or chelating agents produced by the bacteria. Heterotrophic bacteria require organic supplement for growth and energy supply. Among the bacteria, members of the genus Bacillus are most effective in metal solubilization[19].

Siderophores are low molecular weight Fe(III) coordination compounds that are excreted under iron-limiting conditions by microorganisms, particularly bacteria and fungi, to enable accumulation of iron from the environment[20,21]. Although primarily produced as a means of obtaining iron, siderophores are also able to bind other metals such as magnesium, manganese, chromium (III), gallium (III) and radionuclides such as plutonium (IV)[22,23].

Biomethylation of Hg, As, Se, Sn, Te and Pb can be mediated by a range of bacteria under aerobic and anaerobic conditions. Methyl groups are enzymatically transferred to the metal, and the given species may transform a number of different metal(-loid)s. Methylated metal compounds formed by these processes differ in their solubility, volatility and toxicity [23].

Metal Imobilisation

Bioacumulation and **biosorption**. Bacteria can physically remove heavy metals from solution through association of these contaminants with biomass. Bioaccumulation is the retention and concentration of substance within an organism. In bioaccumulation, metals are transported from the outside of the microbial cell trough the cellular membrane, into the cell cytoplasm, where the metals are sequestred[9,24]. Biosorption describes the association of soluble substances with the cell surface. Sorption does not require an active metabolism. The amount of metal biosorbed to the exterior of bacterial

cells often exceeds the amount predicted using information about the charge density of the cell surface[9,25].

Bioprecipitation. Sulfate reduction is an example for the precipitation of metals ions in solution. Sulfate-reducing bacteria form metal sulfides that are insoluble. The stability of these sulfides depends on maintenance of anoxic conditions[7,24], and nutrients are also inevitable. Stimulating sulfate reducion can increase pH also and form metal hydroxides and oxides that precipitate and do not migrate in soil and groundwater[7].

Biooxidation, **bioreduction**. Microorganisms are also known to oxidize and reduce metal contaminants. Mercury and cadmium can be oxidized while arsenic and iron can be reduced by microorganisms. Cr(VI) can be oxidized to Cr(III) that is less mobile and toxic. Bacteria such as *Bacilus subtilis* and SRB in the presence of sulfur can perform this reaction[7].

Bioremediation Technologies

According to the site, bioremediation technologies are divided to:

- ***in-situ*** – are carried out at the place of the contamination,
- ***ex-situ*** – the contaminated matter is taken off from the natural locality and it is consequently processed[26].

Ex situ bioremediation is usually realized on the specific revised place or in the reactor. The pre-treating of contaminated matter increases the efficiency of this process[26]. *Ex-situ* methods have been around longer and are better understood, and they are easier to contain, monitor, and control. However, *in-situ* bioremediation has several advantages over *ex-situ* techniques. *In-situ* treatment is useful for contaminants that are widely dispersed in the environment, present in dilute concentrations, or otherwise inaccessible (e.g., due to the presence of buildings or structures). This approach can be less costly and less disruptive than *ex-situ* treatments because no pumping or excavation is required. Moreover, exposure of site workers to hazardous contaminants during *in-situ* treatment is minimal[27].

Broadly, bioremediation strategies can be further divided into natural attenuation, biostimulation, and bioaugmentation strategies[27].

Bioaugmentation presents an addition of microorganisms or their products, such as biosurfactants or enzymes28. Thus, inoculation of 'specialized' biomass may allow for an increased biodegradation of target pollutants as well as a more effective detoxification of the solid matrix[29] . Another common result of bioaugmentation is the dramatic reduction of remediation times[30,31]. Indigenous or exogenous, standard or modified microorganisms are used[32,33]. Generally, they present mixed cultures of microorganisms, but it could be also pure bacterial strains adapted onto the aimed contaminant in the laboratory[34,35].

Biostimulation can be aggressive or passive, in that electron donors, electron acceptors, and trace nutrients can be injected into the environment to stimulate indigenous organisms to increase biomass or activity to affect the contaminant. Passive biostimulation techniques include simple infiltration galleries or simply spreading fertilizer on surface without any pumping or mixing[25,27].

Natural attenuation relies on the intrinsic bioremediation capabilities of that environment. Environments high in organic carbon and energy sources, low contaminant concentrations, and without significant nutrient deficiencies may be able to degrade or transform the contaminants of concern without any intervention [27].

Conclusions

Environmental biotechnologies with applications of bacteria are eco-friendly and cost effective. They present natural technologies for treatment of toxic metals from soil. The following development is desirable, because of the high specificity and the time-consuming of biological processes and because of the difficulty to control them.

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P62 DISTRIBUTION OF PHTHALIC ACID ESTERS (DEHP, DBP) IN CHICKEN TISSUES AND ORGANS

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Introduction

Phthalates, ubiquitous food and environmental contaminants, are the most frequently used plasticizers for PVC. Due to the widespread use of plasticized PVC for a vast number of technical purposes and for some food contact materials, the phthalates are produced in huge amounts. Several million tons of phthalates are used per year worldwide in the production of soft polyvinyl chloride and other plastics. Phthalates are not chemically bound in to the products and are released continuously into the air or leach from the products[1]. The migration of phthalates from packaging materials containing these compounds to fatty foodstuffs is a well-known source of food contamination[2]. Major and most toxic phthalic acid esters (PAEs) associated with food are di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DBP). PAEs entering the human body are rapidly hydrolyzed to the monoesters and than metabolized and excreted with urine and feces[1].

PAEs are considered highly hazardous to human health because they disrupt the hormonal balance and impair reproduction and development. Some of them are considered as potential carcinogens, teratogens and mutagens. Additionally, phthalates are suspected to trigger asthma and dermal diseases in children. The main objective of the present work is to investigate the real content of DEHP and DBP in samples of chicken's muscle, skin, fat and liver[3].

Experimental

M e t h o d s

Phthalates content measurement in lyophilisated chicken tissues is made in four separate steps, where every sample is analyzed in parallel which brings additional measurement correctness check. Blank is important for glass cleaness check and to specify chromatography background. First step consists of fat extraction from tissues using mixture of organic solvents (acetone : hexane; 1 : 1). In second step, after dissolving co-extracts in mobile phase (dichlormethane: cyclohexane; 1 : 1), they are separated by Bio-Beads S-X3 gel-permeation chromatography. Third step comprise of cleaning of eluate with usage concentrated sulfuric acid. High performance liquid chromatography on Separon SGX C 18 with UV detection at 224 nm was last, fourth step. The mobile phase was acetonitrile – water (1 : 1) pumped at a flow-rate of 1.0 ml min^{-1}. Retention time of DBP was ca. 3.5 min. and DEHP ca 8 min.

M a t e r i a l s

32 chickens ROSS 308 were used in our work. Chickens were separated into four groups of eight Chickens depending on ingredients in feeding mixture. Chickens in second group were fed common feeding mixture fortificated by 3% coleseed oil with low phthalate content into BR2 and 5% into BR3. In this case, vegetative oil was stored in the tin container. Common feeding mixture with 3% coleseed oil with high phthalate content added into BR2 and 5% into BR3 was fed by third group. Coleseed oil for third group was stored in plastic wrapping. The fourth group was comprised by chickens fed by routine feeding mixture with addition 3% animal fat into BR2 and 5% into BR3. 42 days old chickens were butchered and their muscles, skin, fat and liver were used for making samples for analysing. Samples were lyophilisated and before next analysing were hold in freezer in –18 °C. Table I illustrates contents of both phthalates in feeding mixture used in our work.

Table I
Contents of both phthalates in ingredients added to feeding mixture

	DBP [mg kg^{-1}]	DEHP [mg kg^{-1}]	ΣDBP+DEHP [mg kg^{-1}]
Coleseed oil (tin)	15.56	2.25	17.81
Coleseed oil (plastic)	51.35	< 0.20	51.35
Animal fat	43.28	2.10	45.38

Results

Figs. 1.–4. represents chickens separated into four groups groups depending on kind of ingredients in feeding mixture. Each figure expresses average value of contents DEHP and DBP in edible part of chicken's body. Fig. 1. demonstrates real average contents of phthalates in muscle (0.35 mg kg^{-1} DEHP; 0.22 mg kg^{-1} DBP), skin (1.18 mg kg^{-1} DEHP; 0.36 mg kg^{-1} DBP), fat (1.36 mg kg^{-1} DEHP; 0.47 mg kg^{-1} DBP) and liver (0.16 mg kg^{-1} DEHP; 0.02 mg kg^{-1} DBP) in group of chickens fed by common feeding mixture without phthalate addition. Findings in muscle (0.08 mg kg^{-1} DEHP; 0.08 mg kg^{-1} DBP), skin (1.10 mg kg^{-1} DEHP; 0.51 mg kg^{-1} DBP), fat (1.92 mg kg^{-1} DEHP; 0.49 mg kg^{-1} DBP) and liver (0.24 mg kg^{-1} DEHP; 0.11 mg kg^{-1} DBP) of second group is depicted in Fig. 2. Contamination of muscle (0.32 mg kg^{-1} DEHP; 0.15 mg kg^{-1} DBP), skin (1.38 mg kg^{-1} DEHP; 0.57 mg kg^{-1} DBP), fat (3.27 mg kg^{-1} DEHP; 1.28 mg kg^{-1} DBP) and liver (0.16 mg kg^{-1} DEHP; 0.03 mg kg^{-1} DBP) of chickens of third group shows Fig. 3. In fourth group we determined following contents of phthalates: muscles (0.39 mg kg^{-1} DEHP; 0.22 mg kg^{-1} DBP), skin (1.60 mg kg^{-1} DEHP;

0.44 mg kg^{-1} DBP), fat (1.85 mg kg^{-1} DEHP; 0.74 mg kg^{-1} DBP) and liver (0.23 mg kg^{-1} DEHP; 0.13 mg kg^{-1} DBP).

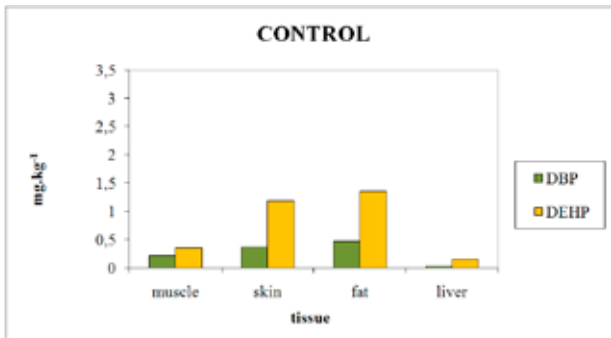

Fig. 1. **First group of chickens fed by common feeding mixture**

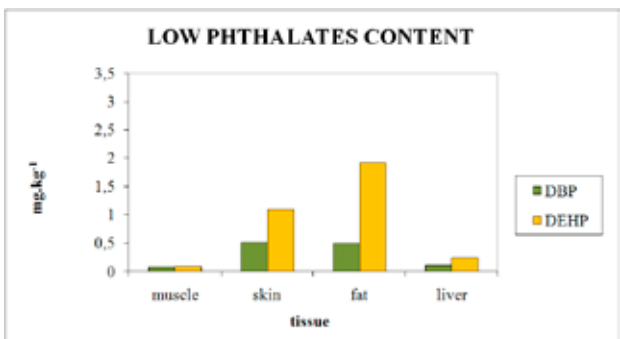

Fig. 2. **Second group of chickens fed by common feeding mixture with addition of coleseed oil with low phthalates content**

Conclusions

Our measurements proved lipophilic character of phthalates, which were accumulated in chicken's fat tissue and skin. That means that fat tissue is useful indicator of phthalates contamination. Liver, despite containing more fat than muscles, had lower values of phthalates mainly because of their enzymatic base, which were transforming phthalates to metabolites. Relatively high contents of phthalates in control group points at ubiquitous content of these contaminants. The values of DEHP and DBP were identically higher in all samples.

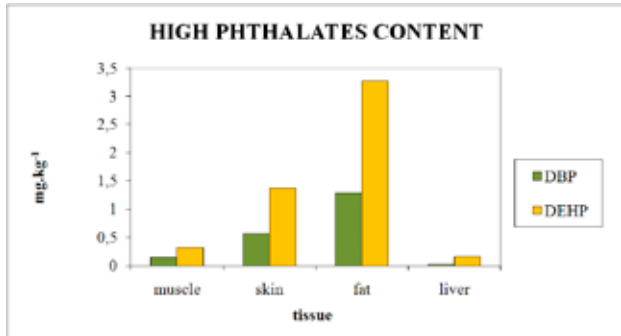

Fig. 3. **Third group of chickens fed by common feeding mixture with addition with coleseed oil with high phthalates content**

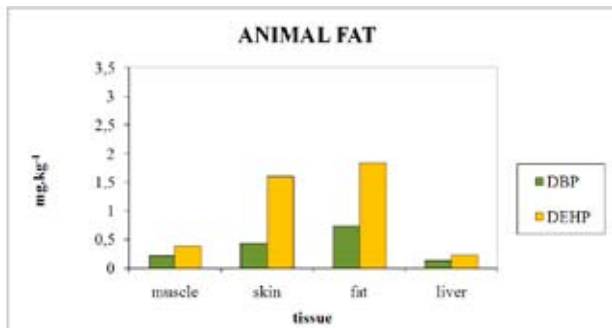

Fig. 4. **Fourth group of chickens fed by common feeding mixture with addition of animal fat**

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P63 PURIFICATION PROCESS INFLUENCE ON THE PAH DETERMINATION IN REAL SOIL SAMPLES

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Introduction

The analytical procedure usually consists of several partial steps: the isolation of analytes, extract purification, and the concentration of monitored analytes[1]. Environmental analysis currently tends to use such analytical procedures to allow the reliable and fast determination of monitored substances at the lowest possible costs. In the field of sample preparation, attention is devoted particularly to the combination of methods which enable the selective extraction of analytes followed by their concentration. The accuracy and precision of measurement in particular are monitored in the instrumental end-point as they are closely linked to the selectivity and sensitivity of analytical instruments. A special emphasis is laid on the confirmation techniques which should prove the results obtained using a typical method and reduce the risk of false-positive and false-negative results[2,3].

Polycyclic aromatic hydrocarbons (PAHs) are formed in the incomplete combustion of organic matter, for example during combustion of fossil fuel or wood, in running engines of motor vehicles, during fire and during free incineration of biomass or municipal waste[4]. They are also produced naturally, for example in active volcanoes.

Individual PAHs differ significantly by their properties which define their potential risks. They are characterized by great variability in terms of toxic, physicochemical and chemical properties that may affect the environment. They produce different effects on individual species and the link between toxicity of individual PAHs and their structure is currently being investigated[5].

Experimental

The effect of the purification procedure on the content of analytes was investigated in samples of soil. The isolation of PAHs from soil was performed using microwave extraction. Samples were purified using column chromatography. HPLC was used as final analytical method.

E x t r a c t i o n

The isolation of PAHs from soil was performed using microwave extraction. The extraction step lasted 25 minutes, at a temperature of 120 °C and an input of 1,200 W, by using 20 ml of a hexane/acetone (1 : 1) mixture. Microwave extraction provided two sets of samples; each set was subsequently subjected to a different purification method.

P u r i f i c a t i o n

We monitored influence of the purification process on the determination of monitored analytes. We carried out comparison of two purification process.

Purification 1: Samples were cleaned-up using column chromatography on a silica column (5 g). The sample was applied on the top of the column; 10 ml of hexane were applied on the top of the column prior to the complete adsorption of the sample on the column, followed by elution with 5 ml of an eluting mixture consisting of hexane : dichloromethane (1 : 1) and finally with 10 ml of the same mixture. The last fraction was collected.

Purification 2: The samples in the second set were purified using column chromatography on a column with silica gel (4 g) and florisil (4 g). The sample was applied directly on the prepared column. The column was washed with 60 ml of a hexane : dichloromethane (1 : 1) mixture prior to the complete adsorption of the sample into the sorbent. The whole eluate was collected.

H P L C

The determination itself was performed using the HPLC Agilent 1100 Series with a DAD and FLD detectors. The HPLC determination was carried out under following conditions: gradient elution (A = 60 % water/40 % acetonitrile; B = 100 % acetonitrile); 0 min A, 30 min B, 40 min. B, 42 min A For separation SUPELCOSIL™ LC-PAH column was used (25 cm × 2.1 mm × 5 μm). The flow-rate of the mobile phase was constant, 0.4 ml min^{-1}, temperature 30 °C. FLD settings were: 0–17.4 min λ_{ex} = 260 nm, λ_{em} = 350 nm; 17.4–42 min λ_{ex} = 260 nm, λ_{em} = 420 nm. DAD setting was: λ = 225 nm.

Results

The main aim of this work was to investigate the effect of the purification process on the content of monitored PAHs in the real samples of soil. For better clarity, the results are provided in graphs and expressed in percentage; for assessment, the values determined in the first set of samples (purification on silica) were chosen as 100 % for assessment purposes. The results are presented in Figs. 1.–3. for individual samples of soil.

It follows from Fig. 1. that both purification techniques provided comparable results. Fig. 2. shows the results for

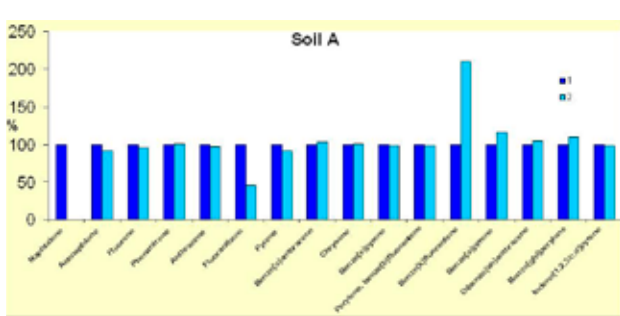

Fig. 1. **Influence of a purification procedure – soil A**

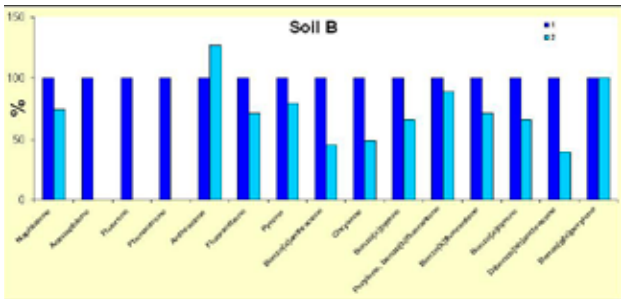

Fig. 2. **Influence of a purification procedure – soil B**

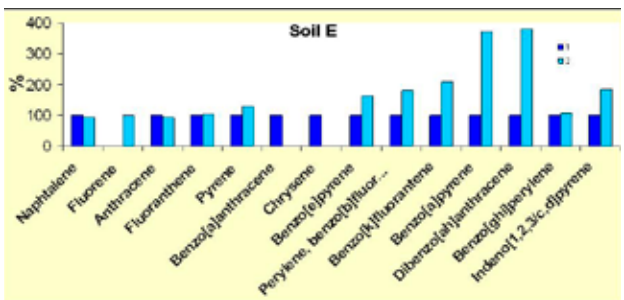

Fig. 3. **Influence of a purification procedure – soil E**

soil B, better results were obtained using the first purification method where the third fraction was the only fraction analysed. Samples of soil C exhibited a common trend for lower-molecular-weight PAHs where the method of extract purification had hardly any effect on the final content in the given matrix. On the contrary, the second purification method using two sorbents (silica gel and florisil) was proven optimal for the PAHs with higher molecular weight.

Conclusions

However, in this context it is important to point out that the yield of pre-analytical techniques is significantly influenced by the sample matrix by the way of matrix effects. The structure and chemical qualities of individual PAH also plays an important role.

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P64 OPTIMALIZATION OF SPME METHOD FOR SPICE CONTENTUAL SUBSTANCES DETERMINATION

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Introduction

The SPME method can be used to extract analytes from different matrices. It has been employed in the isolation of analytes from the air, food, water, and other matrices[1]. The SPME method has a number of major advantages such as fast rate, high sensitivity and good accuracy; the detection limit regularly achieved for the above-mentioned matrices ranges in ng kg^{-1}. First published in 1989, the SPME method is now a well established extraction method whose application has been assessed and documented[2].

The analyses of organic, fragrant, and flavouring components as well as sample preparation are usually started with the concentration of an analyte. If one considers a time demand, the passive SPME sampling technique is the quickest, with sample preparation not exceeding 30 minutes[3].

The consistency of the results and the reliability of detection with SPME as well as repeatability and reproducibility are influenced by a number of factors, like polymer polarity and the thickness of a polymer layer (stationary phase) on the surface of a fibre, the sampling method, the pH, the ionic strength of a solution, sample temperature, agitation, etc.

Experimental

The objective of this paper was the optimization of solid phase microextraction (SPME) method, which is used for determination of the essential oils in spice. Analytes were analysed by GC/MS.

S P M E

The SPME method was investigated with different fibers (polydimethylsiloxane – 100 μm thickness and polydimethylksiloxayne-divinylbenzene – 65 μm), extraction time (1; 2; 5; 10; 15; 20; 30; 40 and 60 minutes), extraction temperature (30; 40 and 50 °C) and incubation of sample (0; 5; 10; 15; 20 minutes).

G C / M S

The determination itself was performed using the GC 6890N (Agilent Technologies, USA) with a mass-spectrometric detector 5973N MSD (Agilent Technologies, USA); for separation HP-5MS capillary column was used (30 m × 0.25 mm × 0.25 μm). The injector temperature was 270 °C, the ion source temperature was 230 °C and the Transfer Line temperature was 250 °C. The temperature programme was 45 °C for period of 2 minutes, 5 °C min^{-1} to 200 °C, 200 °C for period of 2 minutes; the total analysis time was 35 minutes. The flow-rate of the carrier gas (He) was constant, 1 ml min^{-1}.

Results

The aim hereof was to optimise the SPME method for determination of the essential oils in spice. We monitored influence of the stationary phase on the sorption of monitored analytes. We carried out comparison of two PDMS fibres (100 μm) and PDMS-DVB (65 μm). The results obtained are stated in Fig. 1. From it follows that the PDMS-DVB fibre (65 μm) shows higher sorption effectivity for all the monitored pesticides.

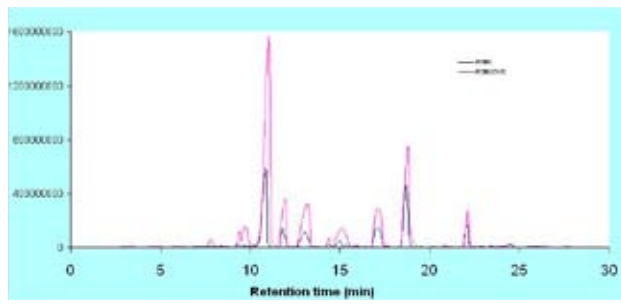

Fig. 1. **Influence of the fibre type on response of detectors for the monitored analytes**

Also the influence of temperature on sorption of the target compounds was monitored. The following temperatures were tested: 30, 40 and 50 °C. Lower-molecular-weight terpenes were shown to have the best responses at a temperature of 30 °C while for the medium-molecular-weight terpenes (around 150 g mol^{-1}), the effect of different temperature on the adsorption of analytes wasn't so strong. Terpenes with the highest molecular weight (204 g mol^{-1}) exhibited the highest response at a temperature of 50 °C. On the basis of the results and literature data, the sorption value at 30 °C was selected.

Various sorption times were measured (1; 2; 5; 10; 15; 20; 30; 40, and 60 minutes) at temperature of 30 °C. The time of 15 minutes seems to be suitable for both the sensitivity and a good repeatability.

This study also investigated the effect of sample conditioning before analyte sorption. The conditions of analysis

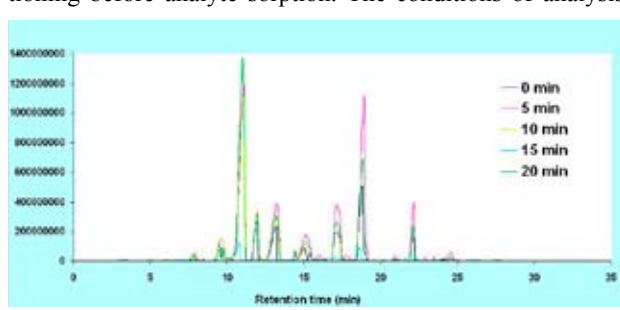

Fig. 2. **Influence of the incubation time on response of detectors for the monitored analytes**

Table I
RSD in determination of analytes

Analyte	RSD	Analyte	RSD
α-pinene	13	menthone	32
camphene	11	borneol	18
sabinene	14	estragol	28
β-pinene	9	α-cubebene	12
β-myrcene	7	thymol acetáte	38
α-phellandrene	10	ylangene	31
3-carene	7	copaene	27
α-terpinene	7	α-bourbonene	34
2-nitro-p-cymene	3	isocaryophyllene	35
limonene	23	β-caryophyllen	26
eucalyptol	8	humulene	31
γ-terpinene	4	α-caryophyllen	36
β-terpineol, cis-	15	isoledene	32
linalool	13	α-muurolene	36
thujone	22	epizonarene	36
camphor	12	karyophyllene oxide	34

were as follows: the duration of sorption was 15 minutes at a temperature of 30 °C. This temperature was also used for sample conditioning. Fig. 2. shows the comparison between the different durations of sample conditioning (0; 5; 10; 15; 20 minutes) and it is seen that the 5-minute conditioning is the most suitable as it gives the highest response for the majority of target analytes.

The repeatability of the method was determined based on 5 repetitions and is expressed as a relative standard deviation (Table I). Terpenes are listed in the table according to their increasing molecular weight. It follows from the results that lower-molecular-weight terpenes have better repeatability than more volatile terpenes with higher molecular weight. Standard deviations vary in a rather wide range of 28–38 % for substances with higher molecular weight which may be attributed to the optimization of the SPME method where some parameters (particularly the duration and temperature of sorption) were chosen at such levels to match for the whole group of 32 monitored substances.

Conclusions

SPME conditions used for optimalization of head-space method were follows: 15 min exposition of PDMS-DVB (65 μm) fiber at 30 °C in head-space above 0.5 g of spice sample in vial. The incubation of sample was 5 minutes at extraction temperature 30 °C.

This work has been supported by by the grants given by Ministry of Education, Youth and Sports of the Czech Republic no. 6215712402.

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P65 DTA AND FLUORESCENCE SPECTRA OF HUMIC ACIDS AS INDICATORS OF HUMAN INFLUENCE ON SOIL

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Introduction

Humic substances (HS) are the most abundant and stable organic C forms found in soil. Based on their solubility in alkaline and acid solutions they can be divided into humic acids (HA), fulvic acids (FA) and humin. Spectroscopic measurements provide valuable information on the nature of soil HS, they are nondestructive, require only small amounts of sample, and are experimentally simple as referred by Stevenson[6]. Thermal analysis curves, heat fluxes, absorption and fluorescence properties can provide useful information on nature, origin, chemistry and structure of HS Blaser et al.[2]; Gonet et al.[3], Senesi et al.[5].

In this study we investigated differences in absorptive, fluorescence and thermal characteristics between humic acids isolated from humus horizons of soils with different land use (agroecosystem and forest ecosystem).

Experimental

Samples were collected from A-horizons of soil pits dug on cultivated and nearby oak-hornbeam forest soils from locality Báb (south-western Slovakia). The parent material was calcareous loess. Samples were compared in two pairs:

- Profiles 1 and 2 – located at an upland position, 80 m apart: 1 – forest soil, Luvic Phaeozem, 2 – cultivated soil, Haplic Chernozem
- Profiles 3 and 4 – located at a colluvial foot slope, 60 m apart: 3 – forest soil, 4 – cultivated soil, both on Orthic Luvisol

Humic acids (HA) were isolated by Orlov[4] method. Total luminescence spectra (TLS) of HA were recorded on a Shimadzu RF5000 spectrometer, and absorption spectra on a Shimadzu UV 240. For measuring, the stock solutions were diluted to organic C concentration 10 mg dm^{-3}, and adjusted to pH 7. TLS excitation slit width was set to 5 nm, emission to 3 nm. TLS were recorded as a series of 10 synchronous scan spectra with differences between excitation and emission wavelength ($\lambda_{ex}/\lambda_{em}$) of 25 nm.

Thermal properties were recorded on Derivatograph C – MOM Hungary. Detailed information concerning differential thermal analysis (DTA) was presented by Gonet et al.[3]. Based on the results, value "Z", proportional to "aliphaticity" of HA was calculated. It expresses the ratio of weight loss in low temperature (endo + exo1) to weight loss in high temperature range (exo2).

Results and Discussion

The absorbance ratios of UV-VIS spectra are listed in Table I. Stevenson[6] stated that UV-VIS spectra of HA are broad, featureless and monotonously decreasing with increasing wavelength, thus absorbance ratios were used in determining their chemical structure. $A_{280/465}$ ratio reflects the proportion between the lignin structures more resistant to humification and materials at the initial stage of transformation, $A_{280/665}$ – ratio between groups resistant to humification and strongly humified ones, $A_{465/665}$ decreases with increasing molecular weight and condensation. In our study, higher values of absorbance ratios indicated that HA from forest soil content more lignin type compounds and had lower degree of aromatic structures condensation than that from the cropped soil.

Table I
Ratios of humic acid absorbances, fluorescence peak positions and intensities

Profile	$A_{280/665}$	$A_{280/465}$	$A_{465/665}$	$\lambda_{ex}/\lambda_{em}$ 455/515 peak intensities [a.u]	$\lambda_{ex}/\lambda_{em}$ 310/480 peak intensities [a.u]
forest, upland	5.25	28.33	5.40	280	245
cultivated, upland	4.53	22.46	4.96	>350	<350
forest, slope	5.06	28.00	5.53	315	280
cultivated, slope	4.67	21.54	4.62	>350	315

Table II
Thermal properties of humic acids

Profile	Area under DTA effects [% of total]			Z
	endo	exo1	exo2	
forest, upland	4.1	57.6	38.3	1.61
cultivated, upland	2.6	35.6	61.8	0.62
forest, slope	4.4	51.6	44.0	1.27
cultivated, slope	5.7	31.3	63.0	0.59

Three-dimensional total luminescence spectra (TLS) enable detailed view with more complete information than simple excitation, emission, or synchronous scan spectra Blaser et al.[2]. TLS of studied HA (Table I) performed two

major peaks, a first at $\lambda_{ex}/\lambda_{em}$ = 455/515 indicating highly conjugated aromatic compounds, like disubstituted coumarins, xanthones and quinones and a second peak at $\lambda_{ex}/\lambda_{em}$ = 310/480 nm indicating simple phenolics like hydroxysubstituted benzoic and cinnamic acid derivatives Senesi et al.[5]. Peaks occured at the same positions, and differed only in fluorescence intensity, which was the highest at $\lambda_{ex}/\lambda_{em}$ = 455/515 nm. This is evidence that HA from cultivated soils were more humified and polycondensed. Alberts et al.[1] reported, that the peak with similar $\lambda_{ex}/\lambda_{em}$ = 455.7/510.6 nm was observed in HA form Hungarian soils.

Thermal properties, mainly the degree of aliphaticity "Z" (Table II) was lower in HA isolated from cultivated soil (0.62 and 0.59) than HA from forest soil (1.61 and 1.27), what confirmed higher condensation of aromatic structures or humification degree of HA from cultivated soil.

Conclusions

Results of the present study confirmed that absorbance, fluorescence and thermal characteristics of HA can be a sensitive index of structural changes between cultivated and natural soils, thus, they can be used as an indicator of anthropogenic influence on soil.

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P66 EVALUATION OF HEAVY METALS MOBILITY IN SEDIMENTS FROM THE HNILEC RIVER, SLOVAKIA

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Introduction

The contribution deals with the content and mobility of heavy metals: copper, nickel and lead in the samples of sediment load from drainage basin of the Hnilec River.

The former mining activities with the following treatment of iron and copper ores have had negative effect on the region of Middle Spiš. The main anthropogenic sources of the contamination of environment come from the localities Krompachy, Rudňany and the surroundings of Spišská Nová Ves, and also the river-basin of the Hnilec, the Ružín No. 1, the Palcmanská Maša reservoirs and an old environmental loads after mining activities in Smolník area. The flooded deposit in Smolník produces heavily mineralized acid waters that contaminate water and sediments of Smolník stream, Hnilec River and even Ružín reservoir. The deposit is not stable and negatively influences the surroundings[1].

Many sequential extraction methods were employed for the determination of particular forms of heavy metals contained in the sediments, differing in the number of the extraction steps, extraction solvents used extraction procedures. Leachate contains a group of elementary forms with similar physical and chemical properties. The extraction process evaluates the strength of bonding of the metal forms to different soil phases, to ion-renewable, carbonated, reducible, oxidable and finally to resistant residue[2]. The best known is the five-step sequential extraction of soil published by Mc Laren and Crawford and Tessier[3]. This method can differentiate heavy metals held in the sediments in five steps. It can be applied to provide a quick indication of changed element mobility in the monitored locality[4].

Experimental

Characteristic of the Sediments

The one-shot sequential extraction procedures were applied on eight sediment samples collected from the industrially polluted region of Central Spiš, Slovakia, localities of the Ružin No. 1 reservoir, the Palcmanská Maša reservoir and stream Smolník situated on the Hnilec River. The reference material of sediment from river Labe was also used for analysis. Sampling was realized in the year 2007 and total content of copper, nickel and lead were determined. Finally, the samples were dried, quartered, sieved under 1 mm and mineralised in a microwave pressure digestion system MWS-3.

Sequential Extraction

The sequential extraction was conducted under the following procedures: **Fraction: A –** released forms were determined in the extract of extraction solvent – 2M HNO_3. The supernatant was filled up to 100 ml. The rest was used in the second step. This fraction contains metals bonded to sulphide and phosphate. **Fraction: B –** potentially mobile forms were determined in the extract of extraction solvent – 0.05M EDTA. This fraction contains metals in ion-changing form and bonded to carbonates. **Fraction: C –** mobile forms were determined in the extract of extraction solvent 0.1M $CaCl_2$. This fraction contains metals in ion-changing form. **Fraction: D –** residual forms presents extracted phase e. g. metals bonded to silicate structure and to a crystal lattice of primary minerals. The residuals were dried at room temperature. After drying and homogenization, the single extracts were measured by the absorption spectrometry method. Copper was determined by flame atomization and nickel and lead by graphite furnace atomic absorption spectrometry (VARIAN, Australia).

Results

Active soil reaction (pH) and oxidation-reduction potential is presented in Table I. It has shown that only sample SP (stream Smolník – mine Pech) was an acid sediment and the rest are slightly alkaline sediments. The increase of pH causes the growth of copper adsorption by colloid, clayey and organic material and thereby the mobility of copper in soil decreases[5].

Table I
The description of sampling places and chemical composition of sediments taken in area of the Hnilec Rriver in 2007

Locality		pH	Redox potential [mV]	Organic portion [%]	Dry basis [%]
Ruzin	VDR1	7.55	259	10.02	36.40
Ruzin	VDR2	7.25	254	10.18	54.52
Ruzin	VDR3	7.52	257	4.50	42.40
Pal. Masa	PM1	7.52	257	18.85	29.54
Pal. Masa	PM2	7.60	259	13.78	34.86
Pal. Masa	PM3	7.73	262	15.89	31.25
Pal. Masa	PM4	8.06	265	18.20	39.9
Smolník	SP	3.3	355	26.01	53.7

The results of copper, zinc, nickel and cadmium content determined in extraction solvents after extraction of sediments using sequential extraction are given in Table II.

The results of sequential extraction are presented as the percentage yield of metal from sequential extraction, where 100 % is total concentration of metal in sediment.

The results in cumulation histogram (Fig. 1.) show that the highest concentrations of copper were determined in the fraction A in released forms and the highest percentage yield in sample VDR1, 2 with values 65.7 and 60.3 % respectively. The yields in the potentially mobile and mobile forms were

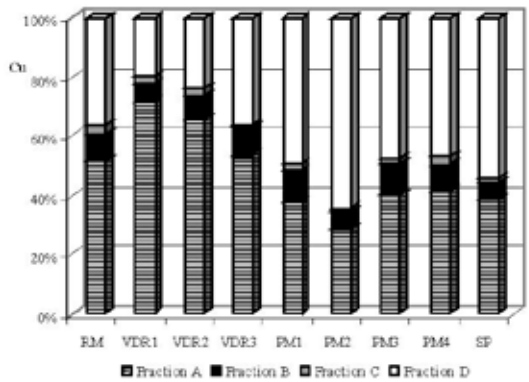

Fig. 1. **Cumulation histogram of the copper yields in fractions A–D of the sequential extracts of the sediments, RM – Reference Material-sediment, VDR1 – 3 Ružin No. 1 reservoir, PM1–4 Palcmanska Masa reservoir, SP – stream Smolník, mine Pech**

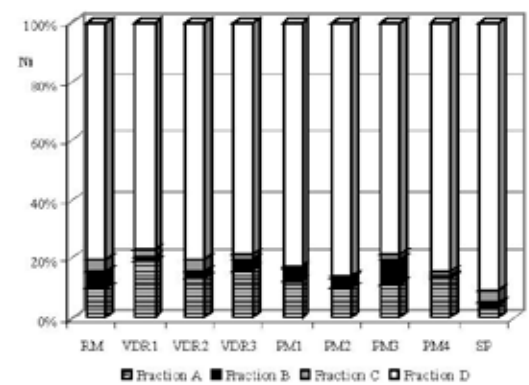

Fig. 2. **Cumulation histogram of the nickel yields in fractions A–D of the sequential extracts of the sediments, RM – Reference Material-sediment, VDR1 – 3 Ružin No. 1 reservoir, PM1–4 Palcmanska Masa reservoir, SP – stream Smolník, mine Pech**

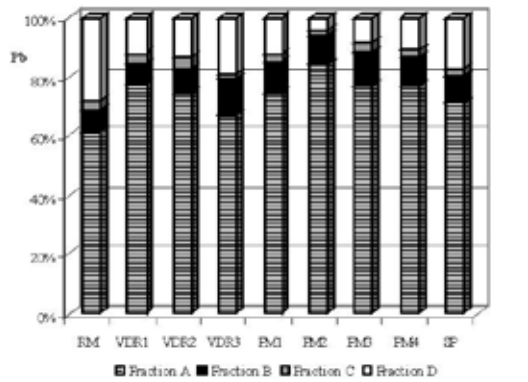

Fig. 3. **Cumulation histogram of the lead yields in fractions A–D of the sequential extracts of the sediments, RM – Reference Material-sediment, VDR1 – 3 Ružin No. 1 reservoir, PM1–4 Palcmanska Masa reservoir, SP – stream Smolník, mine Pech**

low. In fraction D the highest values of yield were confirmed in samples PM2 64.1 % and SM 55.2 %.

The Fig. 2. presents the distribution of nickel in fractions. The highest concentration of nickel was determined in fraction D, where the highest yield was in sample PM4 80 % and the least in sample SP 16 %. In the other forms, the nickel content in yield was very low.

According to Fig. 3., the highest concentrations of lead were found in fraction A, where the highest values of yield were in samples PM 3 64.2 % and PM 2 – 1 (54.4–51.6 %). In fraction B–C, the content of was in low percentage yield and the yield was higher in fraction D.

The results of yield are given in Table III, where the total content of particular heavy metals Cu, Ni, Pb in sediments were compared with the sum of metal concentrations in fraction A–D from sequential extraction. In sample PM2, 100 % yield of copper was determined.

Table II
Copper, nickel and lead content determined in extraction solvents after extraction of sediments using sequential extraction method, RM – Reference Material-sediment, VDR1 – 3 Ružin No. 1 reservoir, PM1–4 Palcmanska Masa reservoir, SP – stream Smolník, mine Pech

Fraction	Element	Sediment contents [mg kg^{-1}]								
		RM	VDR1	VDR2	VDR3	PM1	PM2	PM3	PM4	SP
A	Cu	33.5	160.0	178.2	166.0	14.6	10.0	19.6	18.4	216.0
	Ni	3.3	9.6	4.6	5.7	7.0	5.1	7.4	4.5	0.3
	Pb	33.9	34.7	40.4	35.3	26.3	55.5	18.0	17.1	34.9
B	Cu	6.0	13.4	22.6	29.4	4.0	1.8	5.0	4.0	35.0
	Ni	2.0	0.6	07	1.3	2.4	1.4	5.4	5.0	0.1
	Pb	4.1	2.9	4.2	6.4	3.7	6.7	2.7	2.1	4.4
C	Cu	2.0	5.2	6.8	2.8	0.8	0.5	1.0	1.2	6.2
	Ni	1.3	1.2	1.6	0.6	0.6	0.4	1.1	1.4	0.3
	Pb	1.7	1.6	2.3	0.8	0.8	1.1	0.8	0.6	1.2
D	Cu	23.7	45.2	64.2	11.2	18.9	22.0	0.3	20.8	306
	Ni	26.7	38.5	27.3	27.4	47.2	42.6	51.3	47.4	7.1
	Pb	15.6	5.4	7.3	9.8	4.4	2.7	2	2.4	8.7

Table III
Analysis comparison of total dissociation and the sum of metal concentrations in the sequential extraction procedure of the sediment samples

Element		Sediment [mg kg^{-1}]								
		RM	VDR1	VDR2	VDR3	PM1	PM2	PM3	PM4	SP
Cu	Sum of ex.st.	65.2	223.8	271.8	309.4	38.3	34.3	48.6	44.4	563.2
	Total cont.	79.9	243.5	295.5	353.9	45.8	34.3	49.5	47.9	572.0
	Yield %	81.6	91.9	92.0	87.4	83.6	100	98.2	92.7	98.5
Ni	Sum of ex.st.	33.3	49.9	34.2	35.0	57.2	49.5	65.2	58.7	7.8
	Total cont.	35.5	66.0	82.3	67.4	79.6	60.1	80.0	59.2	44.2
	Yield %	93.8	75.6	41.5	51.9	71.8	82.4	81.5	98.5	17.6
Pb	Sum of ex.st.	55.3	44.6	54.2	52.3	35.2	66	23.5	22.2	49.2
	Total cont.	77.6	108	121	80	51	102	28	37	92
	Yield %	71.3	41.3	44.8	65.4	69	64.7	83.9	60	53.5

The comparison of the sum of metal concentrations in the sequential extraction procedure with of total dissociation verified the suitability of the used method. Moreover, the RM LGC River Sediment was applied, where metal contents are copper – 83. 6 mg kg^{-1}, zinc – 439 mg kg^{-1}, nickel – 34.7 mg kg^{-1} and cadmium – 2.7 mg kg^{-1}.

Conclusions

The one-shot sequential extraction, which characterise the individual forms of heavy metals bonds in the sediments, was used for the determination of heavy metals mobility in the contaminated sediments from the Hnilec River (Slovakia) area. 2M HNO_3 was used to define the released content including different fractions of elements according to their solubility. Potentially mobile and mobile forms represent the sum of risk element forms for the evaluation of sediment biotoxicity. In this connection, the sediments had low content of copper, nickel and lead in potentially mobile and mobile forms (fractions B–C). This fraction contains metals in ion-changing form and bond to carbonates.

Lead and copper reached higher values and nickel was in the least concentration in fraction A (realised forms) in order Pb > Cu > Ni. This fraction contains metals bonded to sulphide and phosphate.

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P67 ELIMINATION OF SULPHATES FROM WASTE WATER OF OLD MINING LOADS

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Introduction

Heavy industry and the impact of mining, although being in decay, significantly facilitate the deterioration of quality of surface waters. Besides the technological water from the existing plants, there is a huge amount of secondarily polluted mining waters due to the rainfall. The rainfall water in the underground areas in old mining works gradually chemically and chemically and biologically reacts with the surrounding rock environment and brings amounts of harmful substances to the surface that pollute the surface waters. They include mainly heavy metals, sulphates, chlorides, phosphates and other substances.

From the point of view of quality of surface waters the area in the vicinity of the municipality of Smolník has been known as one of the worst in Slovakia for a longer period of time and the brook of Smolník is ranked in the worst – the fifth degree of quality as a highly polluted brook.

This work aims at the following: study the possibility of reduction of the concentration of the content of sulphates and other heavy metals set by the EU directives in the mining waters flowing from the old mining load of the deposit of Smolník.

Table I
Trend of mine water quality from Smolník in period from 1986 to 2004[2]

Year	SO_4^{2-} [mg dm^{-3}]	Fe [mg dm^{-3}]	Cu [mg dm^{-3}]	Mn [mg dm^{-3}]
1986	6,004	72.0	37.2	43.3
1987	4,634	51.2	39.4	65.8
1991	1,155	7.0	11.1	12.3
1992	1,233	19.9	10.7	13.5
1993	1,481	61.3	5.3	12.8
19941	1,350	57.8	7.2	11.3
19942	4,000	155.0	11.8	11.0
19943	9,512	914.7	51.3	104.7
1995	5,825	772.4	7.4	136.2
1997	4,133	421.4	3.9	37.2
2000	4,170	137.6	4.3	41.3
2001	3,461	556.0	2.8	37.9
2002	2,296	620.0	1.7	38.4
2003	2,680	501.0	1.5	33.4
2004	2,723	425.0	1.5	27.9

Physical and Chemical Characteristics of the Mining Water from the Deposit of Smolník

Qualitative parameters of the running mining water were monitored systematically. The values often move significantly. They depend on the amount of rainfall, rainfall intensity, period of being held in the mining areas, place (depth), etc. A large number of floods and mainly fires in the mine caused huge increases of presence of Fe and Cu in the mining water. The mining fire by spontaneous combustion of pyrite in 1896–97 caused the increase of the Cu concentration up to 150 g dm^{-3}, in 1910 the Cu concentration increased even to 180 g dm–3 due to the fire! In 1923 57.6 tonnes of copper flew into the recepient.[1]

Table I evidently shows the gradual increase, culmination and finally the decrease of value of almost all indicators. Those changes were caused due to flooding and its completion. After almost four-year exposition of mining waters in the rock environment a strong mineralisation occurred and concentration of all components exceeded all permissible concentrations (they are exceeded today as well).

Elimination of Sulphates using Precipitation and Al-Ions.

The concentration of sulphate ions in the tested water was 2,984 mg dm^{-3} and pH 3.34. When making experiments with the mining water a HACH spectrophotometer, type DR 2000 and a digital pH-meter WTW 330i were at our disposal.

Precipitation of SO_4^{2-} Ions Only at Presence of $Ca(OH)_2$ Without Al^{3+} Ions

Methodological procedure of precipitation:

- $Ca(OH)_2$ – 15 minutes of mixing at 200 rpm,
- suspension filtering,
- determination of the content of sulphates and the pH value (see in Table II).

Table II
Results of mine water desulphatation by $Ca(OH)_2$, Pech pit

Sample no.	$Ca(OH)_2$ [mg dm^{-3}]	pH	SO_4^{2-} [mg dm^{-3}]	Effect des. [%]
1	6	12.59	2,400	19.6
2	8	12.60	2,362	20.8
3	10	12.61	2,385	20.1
untreated water	0	3.34	2,984	–

As the required pH value is 12.4 and more, Table II shows that the sufficient amount of $Ca(OH)_2$ is 5–6 g dm^{-3}. Higher amounts do not impact the pH values any longer. At the same time, one can observe that adding of lime itself has no significant impact on the reduction of the content of sulphates either. Out of the original approximate amount of 2,984 g only 600 g were precipitated and that constitutes the efficiency of only about 20 %.

Precipitation of SO_4^{2-} Ions at Presence of $Ca(OH)_2$ and Sodium Aluminate (ALR-F)

Methodological procedure of precipitation is based on long range experiments of the scientific group from Technical university in Ostrava[3]: Best results of sulphates precipitation were obtained at pH value close to 12.5.

- $Ca(OH)_2$ – 15 minutes of mixing at 200 rpm
- ALR-F –30 minutes of mixing at 200 rpm
- suspension filtering
- determination of the content of sulphates and the pH value

Al ions changes to form hydrated calcium aluminates and part of arisen mineral ettringite, Fe ions change from bivalent to trivalent ferrous hydroxide and also remain in the mud.

The results of those experiments are shown in Table III[5].

Table III
Results of mine water desulphatation from Pech pit at optimal reagents dosing[4]

Sample No.:	$Ca(OH)_2$ [g dm^{-3}]	ALR-F [ml dm^{-3}]	pH	SO_4^{2-} [mg dm^{-3}]	Effect desul. [%]
1	5.2	3.2	12.56	118.28	96.03
2	5.2	3.4	12.56	199.60	93.31
3	5.2	3.6	12.56	49.92	98.32
4	5.2	3.8	12.56	45.28	98.48
5	5.4	3.2	12.56	64.40	97.84
6	5.4	3.4	12.56	123.28	95.87
7	5.4	3.6	12.55	65.00	97.82
8	5.4	3.8	12.55	49.64	98.33
9	5.6	3.2	12.58	114.40	96.17
10	5.6	3.4	12.58	42.96	98.56
11	5.6	3.6	12.58	0.48	99.98
12	5.6	3.8	12.58	0.97	99.97

Elimination of Sulphates Using Sorbents and Ion Exchangers

As opposed to the static regime when a sorbent or precipitation reagents are added to the water container when they are slowly mixed for a certain optimal time, in case of a dynamic regime the treated water continuously flows through the column the bed of which consists of a sorbent, a ion exchanger or a mixture with the sorbent. Experiments with the mining water from the shaft of Pech – Smolník were performed. The water was stabilised in the ratio of 5 ml HNO_3 (2M) per 1 litre. Elimination of sulphates can be realised using the following methods:

- raw stabilised mining water,
- sorbent – active coal,
- ion exchanger – mixture 50 : 50 catex and anex,
- sorbent – zeolite (Nižný Hrabovec) mineral form –clinoptilolite, structure - tectosilicate, empiric formula – $(Ca,K_2,Na_2,Mg)_4Al_8Si_{40}O_{96} \cdot 24H_2O$,
- mixture of 80 % of zeolite, 20 % cent of A–C,
- mixture 80 %of zeolite, 20 % of C–A,
- ion exchanger – cation exchanger – catex-strog acid Ostion,
- ion exchanger – anion exchanger – anex – light basic annex with styrene-DVB skeleton – Amberlite IRA-96,
- sorbent – slovakite (inorganic composite sorbent) – it is sorbent based on advantages of various minerals properties.

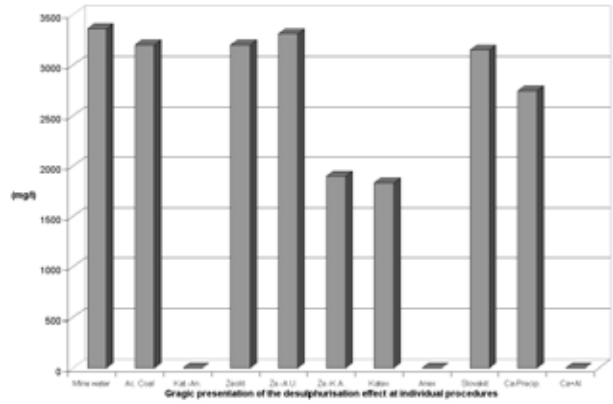

Fig. 1. **Graphic presentation of the desulphurisation effect at individual procedures**

Discussion to the Measured Results.

Sulphates reduction is possible by various methods, like reversing osmosis, electrodialysis, ultrafiltration, diaphragm processes, but these methods are too expensive. Biological methods are long-lasting, that is why we used chemical processes, i.e. sorption by active coal, various sorbents and ion exchangers and precipitation by Al and Ca ions.

Based on the results shown in Fig. 1. the success rate of individual reagents can be definitely set. The inorganic component sorbent slovakite may be considered as relatively successful. It was tested in the static regime as well and it was rather unsuccessful there. The result was surprising in case of the dynamic sorption. The efficiency of elimination of sulphates reached approximately 60 %. The efficiency of 43 % in case of the mixture of zeolite and the cation exchanger and the anion exchanger was caused only due to the small amount of the anion exchanger in the reagent. There was no practical effect of zeolite itself. Almost 100% efficiency was achieved by means of the anion exchanger what is in case of anion elimination logical[6].

Conclusion

As the strict limits for waters discharged to the surface flows set by the European Union begin to come into force also in the recently acceded EU countries the issue of waters discharged from the old mining load of Smolník will have to be dealt with seriously very soon. Several options are offered. The use of ion exchangers would be probably very costly although a very efficient solution. The most probable solu-

tion will be the use of physical and chemical methods, and namely their different combinations, such as neutralisation, precipitation by means of Al ions or sorption by means of composite sorbents.

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P68 ARSENIC REMOVAL FROM WATER BY SYNTHETIC AKAGANEITE

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Introduction

Arsenic is considered one of the most toxic contaminants found in water-streams. It poses serious health risks to humans (e.g. cancer, cardiovascular and neurological effects). There are two main groups of arsenic pollution sources; natural (dissolution of As containing mineral ores) and anthropogenic sources (e.g. arsenic-based insecticides and pesticides, fertilizers, coal combustion, mining, semiconductor industies). The maximum contaminant level of arsenic in drinking water (both in EU and USA) is 10 μg dm^{-3}.

Arsenic has several oxidation states (–3, 0, +3, +5) but the most common forms in natural waters are trivalent arsenite [As (III)] and pentavalent arsenate [As (V)]. The pH and the redox potential (Eh) of the aqueous system determine the predominant form of As; As (III) is dominant in reducing conditions while As (V) in oxidizing conditions. Generally, As (III) is considered to be more toxic than As (V)[1].

There are various methods for arsenic removal from water streams, such as sorption, ion-exchange, precipitation, coagulation and flocculation, reverse osmosis, membrane technologies, electrodialysis, biological processes as well as lime softening. A good overview[2] and a critical review[3] of the available methods are given recently. An effective and commonly used method for water treatment is sorption of arsenic on natural or synthetic sorbents. The most commonly used sorbents can be classified in two main groups: *(i)* sorbents based on iron compounds, which are the most frequently used (e.g. several iron (III) oxides/hydroxides, materials based on iron oxides/hydroxides, natural iron ores and waste materials containing iron particles) and *(ii)* sorbents based on aluminium compounds (e.g. activated alumina or gibbsite). Several other sorbents (clays, manganese dioxide, activated carbon, ion-exchange resins, biosorbents, etc.) have also been studied for As removal[2].

Experimental

Reagents

Analytical grade chemicals were used in all experiments. Model solutions were prepared by dissolving $AsHNa_2O_4 \cdot 7H_2O$ in deionized water, 0.01 M $NaNO_3$ and 0.1 M $NaNO_3$. The pH of the solutions was adjusted with suitable concentrations of NaOH and HNO_3.

Preparation and Characterization of Sorbent

Synthetic akaganeite was prepared by hydrolysis of partially neutralized $FeCl_3$ by addition of NaOH. The precipitate thus obtained was centrifuged and it was subsequently submitted to dialysis to remove Cl^- ions. The material was dried and used for arsenic sorption. XRD analysis[4] confirmed that the material produced was akaganeite. The specific surface area (measured by BET) was 151.3 m^2 g^{-1}.

Sorption Experiments

The effect of pH, initial arsenic concentration, sorbent dose and temperature as well as ionic strength effect at arsenic sorption were studied in batch type experiments. The experiments were performed in a rotary shaker set at 30 rpm and equilibrium time 24 hours. Preliminary experiments have shown that equilibrium was established. The arsenic quantity in solutions was determined by AAS and spectrophotometry before and after the sorption experiments. The sorption capacity (Q) of akaganeite was calculated using the equation:

$$Q = \frac{C_0 - C_{eq}}{C_s} \qquad (1)$$

where C_0 and C_{eq} are the initial and equilibrium arsenic concentration, respectively and C_s is the sorbent concentration in solution.

Results

Effect of Solution pH

It is well known that the solution pH plays an important role in all sorption experiments in aqueous systems. It determines the aquatic chemistry of the system under study (in this case As speciation) and also the charge density of the solid surface (sorbent). It is related to the sorption mechanisms and reflects the nature of the physicochemical interactions of the species in solution and the active sites on the sorbent[5].

The effect of solution pH on As (V) sorption on akaganeite was examined at pH values 2.0–9.0 at ambient temperature. Sorbent concentration in solution was 2 g dm^{-3} and initial arsenic concentration was 100 mg dm^{-3}. The results are given in Fig. 1. for various electrolyte ($NaNO_3$) concentrations. The best sorption is observed at pH 2.0. However, in this case, akaganeite is dissolved and iron was determined in filtrates. For this reason, all sorption experiments were carried out at pH 3.5, where no iron was found in solution. At this pH, the sorption capacity was a bit smaller than at pH 2.0 and around 40 mg As (V) g^{-1} of sorbent. This is considered quite good compared to the average value reported in the literature[3]. The effect of the ionic strength was also studied by adding 0.01 and 0.1 M $NaNO_3$. It is shown that (Fig. 1.) the

effect can be considered as negligible. In a previous study[4] the zeta potential of akaganeite was studied and the point of zero charge was found at pH 7.5. At pH 3.5, the zeta potential was around +20 mV and the predominant As (V) species is $H_2AsO_4^-$. So, attractive electrostatic forces favour sorption. However, at pH 8.0, the zeta potential is negative and the predominant species is $HAsO_4^{-2}$. Electrostatic forces are repulsive but still sorption is achieved due to the existence of specific forces. Calculations with Geochemist Workbench (data not shown here) show that in the presence of iron mineral As (V) can form scorodite (iron arsenate) on the mineral surface and the maximum is obtained at pH 2.0, This coincides very well with the maximum sorption at pH 2.0 in our experiments (Fig. 1.). The existence of scorodite on akaganeite surface was also proved by FTIR spectra[6]. The chemical nature of the sorption can also explain the sorption of As (V) at pH 8.0. In our results, the effect of ionic strength was negligible. However, in the literature[6] a significant effect was observed, but with KNO_3 and in smaller sorbent concentrations (<2 g dm^{-3}). The effect was decreasing with increasing sorbent concentrations. This was attributed to the elimination of the negative charge of akaganeite by the potassium cations and the easier approach of As (V) species to the surface of the sorbent. It is well known that the increase of the ionic strength decreases the effective radius of the electric double layer. The arsenate species can come closer to the surface so that the attractive chemical forces overcome the electrostatic repulsion forces and sorption is achieved.

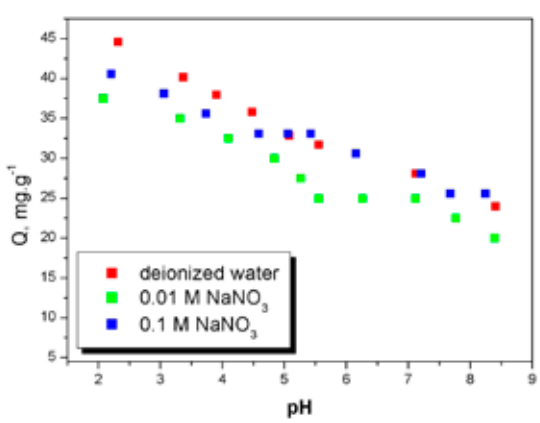

Fig. 1. **Effect of solution pH on As (V) sorption**

Effect of Initial Arsenic Concentration

The effect of initial arsenic concentration (20–200 mg dm^{-3}) was studied with 2 g dm^{-3} sorbent concentration, ambient temperature and pH values 3.5 and 7.0. The results are given in Fig. 2. (data points: experimental data, lines: isotherms). Sorption isotherms were evaluated (from experimental data) using the Freundlich model:

$$Q = KC_{eq}^{b} \quad (2)$$

where Q is amount sorbed per unit mass of sorbent, K and b are constants and C_{eq} is the equilibrium concentration. Freundlich sorption parameters (K, b, R^2) were calculated and are given in Table I. The maximum sorption capacity was around 53 and 37 mg g^{-1} for pH values 3.5 and 7.0 respectively. The R^2 values show a good agreement of the model to the experimental data. As is expected, the K value (which shows the affinity of akaganeite to As (V)) decreases with the increase of the pH.

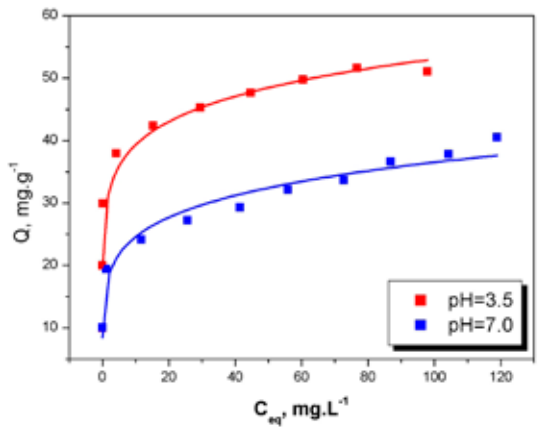

Fig. 2. **Sorption isotherms**

Table I
Freundlich adsorption parameters

pH	Freundlich parameters		
	K	b	R^2
3.0	32.26	0.10	0.98
7.5	12.84	0.23	0.97

Effect of temperature

The effect of temperature on arsenic sorption was investigated at initial arsenic concentration 100 mg dm^{-3}, sorbent concentration 2 g dm^{-3} and 4 different temperatures (ambient, 30, 40 and 50 °C). The experimental results are presented in Table III. The maximum sorption capacity increased with increasing temperatures indicating an endothermic reaction[6]. The maximum sorption capacity (at 50 °C) was around 43 mg g^{-1}.

Table II
The effect of temperature

T [°C]	Q [mg g^{-1}]
ambient temperature	37
30	38
40	40
50	43

Effect of Sorbent Dose

The effect of sorbent dose was studied at ambient temperature, initial arsenic concentration 100 mg dm^{-3} and sorbent

concentration range 0.5–10 g dm^{-3}. The results are presented in Table II. Increase of the sorbent dose in solution caused a decrease of akaganeite sorption capacity. However, this is not a good indicator of sorption ability, because with bigger sorbent concentrations all the available active sorption sites are not occupied. It is important to see what is the remaining As (V) concentration after treatment. In a real system this is the important factor; the remaining concentration should be below the limits. In the last column of Table III the remaining concentrations are given. It is observed a significant decrease of the remaining As (V) conc. with increase of the sorbent dose. It seems that a good dose is 5 g dm^{-3}, which gives a 96 % removal and a satisfactory final As concentration of 4 mg dm^{-3}.

Table III
The effect of sorbent dose

C_S [g dm^{-3}]	Q [mg g^{-1}]	C_{eq} [mg dm^{-3}]
0.5	43.0	78.5
1.0	41.4	58.6
2.0	36.3	27.4
5.0	19.2	4.0
10.0	9.6	4.0

Conclusions

Synthetic akaganeite is a suitable sorbent for arsenic removal, especially at acidic environment. It is a low cost sorbent, as it is prepared from easily available cheap inorganic materials and has a good efficiency (above the average reported in the literature). It removes arsenic by chemical sorption favored by an increase of temperature and the best results are obtained at pH 3.5. However, at pH 7.0, gives also satisfactory results. This is important for the treatment of drinking water. The capacity of the material was not affected significantly with increased ionic strength (up to 0.1 M $NaNO_3$). The experimental data follow (with good agreement) the Freundlich isotherm.

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P69 THE EPR STUDY OF PARTICULATE MATTER

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Introduction

In recent years the role of particulate matter in environmental and health areas has come under increased attention of experts and public. The PM_{10} and $PM_{2.5}$ (particle fractions related to their size) concentrations show higher correlation to the negative health effects in comparison to the total suspended particulate matter[1]. Building constructions and materials play an important role in indoor particulate matter contamination due to particles infiltration through joint construction leakages and material distribution and deposition processes[2].

The dust particles are dangerous not only because of their high concentrations in dependence on their particle size but also because of their nature[3]. The surface of the inhaled particles which determines the biological response is large. The surface reactivity of particles depends on the number of reactive spots on the dust particles surface and on the distribution of various sites (free radicals and paramagnetic species) at the surface[4]. It determines the pathogenic potential of inhaled particles.

This work is concerned with the particulate matter reactivity investigation by concentration of surface active centres.

Experimental

Both total suspended particles (TSP) and PM_{10} measurement includes integral sampling onto a collection material (membrane filter Synpor 0.8 μm pore size, 35 mm in diameter) by sampling equipment VPS 2000 (Envitech, Trenčín) at flow rate of 960 $dm^3 h^{-1}$ during sampling period of approximately 24 hours.

The surface reactive spots concentrations of suspended particulate matter were detected on the spectrometer ERS 220 (GAS, Berlin) with a resonator RSX 216 at room temperature under following conditions: the microwave power 10 mW, modulation amplitude 0.02 mT, time constant 0.5 s. The relative intensity of the signals measured for investigated dust samples was determined by comparing them with those of Mn(II)/ZnS a Cr(III)/MgO as standard samples. The EPR spectrum was recorded as the first derivation of the absorption spectrum at the speed record 14.3 $mT\,min^{-1}$.

The quantitative and qualitative interpretation of obtained spectra was performed by computer software Origin.

Results

Typical EPR spectra of investigated total suspended particulate mater and PM_{10} samples are illustrated in Figs. 1.–3.

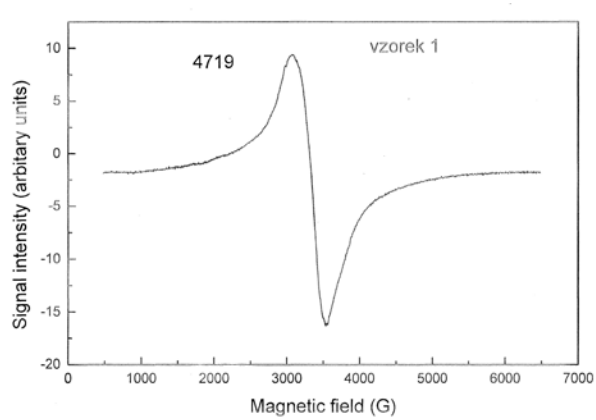

Fig. 1. **EPR spectra of total suspended particulates**

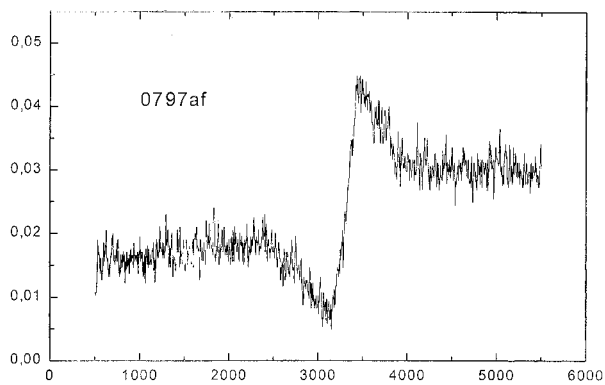

Fig. 2. **EPR spectrum of PM_{10}**

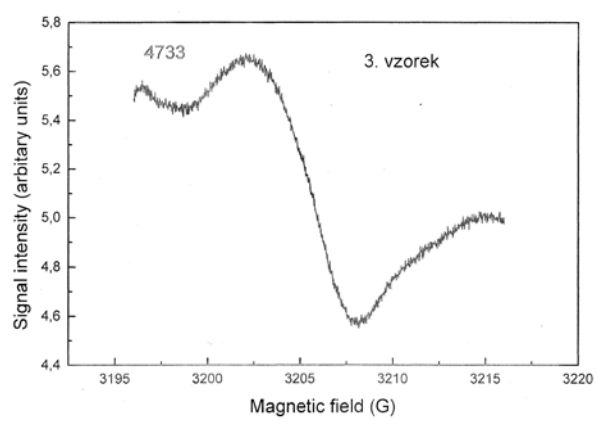

Fig. 3. **Detail of PM_{10} EPR spectrum**

Wide singlet peak centered at g factor value was identified in EPR spectra of particulates. The g factors range from 2.010 to 2.093 for measured particle samples as the Table I shows.

Singlet peak superponed by triplet peak with factor values $g_1 = 2.1162$, $g_2 = 2.0700$, $g_3 = 2.0191$ was identified in EPR spectrum of total particulate matter in Fig. 1. It could be an isotropic triplet (g = 2.07) with splitting constant a = 7.6375 +/ 0.5495 mT.

Table I
G-factor values, ΔH_{pp} and spin concentrations

Sample	g-factor	ΔH_{pp} [mT]	Spin concentration [spin mg^{-1}]
TSP	2.070	34.6	3.84×10^{18}
TSP	2.015	40.0	3.68×10^{17}
TSP	2.020	40.0	1.64×10^{18}
TSP	2.010	40.0	2.22×10^{18}
PM10	2.093	472	2.01×10^{18}
PM10	2.080	554.9	2.02×10^{17}
PM10	2.055	410.8	2.06×10^{17}
PM10	2.070	345.65	1.09×10^{19}

The signals on EPR spectra recorded on the particulate samples were identified as a superposition of signals arising from various paramagnetic species. It is mainly the presence of transition metal ions in a low oxidation state, typically iron, which have to be regard as a possible cause of toxicity. In accordance with results obtained in paper[4], the appearance of Fe^{2+} on the surface of the dust particles and its subsequent oxidation to Fe^{3+} in octaedric configuration leads to production of free radicals at solid-liquid interphase by direct reduction of atmospheric molecular oxygen. These sites may be implicated in the formation of dangerous active oxygen species in vivo.

The total surface spin concentrations of particulate samples were determined in range from 3.68×10^{17} to 3.84×10^{18} spin.g^{-1} for total suspended particulate matter and from 2.02×10^{17} to 1.09×10^{19} spin g^{-1} for PM_{10}. The significant difference between spin concentrations values of total suspended particulate matter and PM_{10} was not found. The determined spin concentrations values are approximately 10^2 times higher than spin concentrations values of mechanically activated quartz and silicon powders[5].

Conclusions

The determined surface spin concentrations obtained from EPR spectra were high. But based on these experimental results of paramagnetic centres occurrence study explicitly, it cannot be expressily considered particulate matter to have toxic properties. The paramagnetic ions testing in special chemical reactions[6] used for this purpose may more accurately indicate potential risk of particulate matter toxicity.

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P70 SELECTION OF PACKING MATERIALS FOR BIOFILTER DEVELOPMENT

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Introduction

Natural materials that are available in large quantities may have potential as inexpensive sorbents. Cost effective alternative technologies or sorbents for treatment of metals contaminated waste streams are needed. A study[1] reported that zeolites, clinoptilolite in particular, demonstrate strong affinity for Pb and other heavy metals. Adsorbing Cd and Zn examined by both modifications with natural bentonite[2]. Results showed that the acid-treatment decreased the adsorption capacity, while the heat treatment did improve capacity. Retention of Pb and Zn on pure calcite was the subject of a number of investigations[3,4]. On the contrary, the number of sorption studies of both ions on magnesite is very limited.

Investigations on the biosorption mechanism of heavy metals show that the metal ions are deposited by adsorption to the functional groups present on the cell wall. Dead as well as living cells are used in the removal of metal ions[5,6]. The batch adsorption experiments demonstrate that the surface complexation approach can be used successfully to quantify the adsorption of Cd in a mixed *B. subtilis* – quartz system as functions of both pH and solute/sorbent ratios[7].

We are interested in the surface complexation approach of zeolite, bentonite, calcite, magnezite and also these materials with bacteria in the behavior various heavy metals in the batch experiments. The objectives of this work were to determine the differences of mineral/water and mineral-bacteria/water interface in sorption capacities of metals.

Experimental

The biosorption experiments were carried out in Erlenmeyer flasks which contained 10 g samples and 100 ml medium. The medium contained (per liter) 0.5 g NaH_2PO_4, 1.0 g $(NH_4)_2SO_4$, 0. g NaCl, and 2 g glucose. The flasks were inoculated with a mixture of *Bacillus cereus* and *B. megaterium* (0.1 g wet bacteria dm^{-3}) that had been previously isolated from Horná Prievrana. The two strains were purified by heat treatment at 80 °C for 15 min followed by streak plating on nutrient agar cultures. The isolates were identified with the BBL Crystal Identification System (Becton, Dickinson and Co., Franklin Lakes, NJ). For identification, the isolates were cultivated on Columbia agar plates per manufacturer's instructions.

The flasks were incubated under dynamic conditions (150 rev min^{-1}) for 3 hours at 25 °C. The liquid phase was contained individual metals in 0.5mM concentration in the forms $ZnSO_4$, $CuSO_4$, $PbCO_3$. The spent media (leachates) were sampled for metal analysis. The chemical controls did not receive an inoculum but were incubated under otherwise similar conditions.

Solid residues were analyzed by X-ray diffraction using a Philips X'Pert SW–binary diffractometer with CuKα radiation (40 kV, 50 mA), equipped with an automatic divergence slit, sample spinner, and a graphite secondary monochromator. Data were collected for 2–60 °2Θ with a step width of 0.05 ° and a counting time of 30 s per 0.05 °. The mineralogy has been evaluated in quantitative terms from X-ray powder diffraction patterns using a Rietveld-based data processing technique.

Quantitative changes in the liquid phase were measured with a Model 30 Varian atomic absorption spectrometer (Varian, Inc., Melbourne, Vic., Australia).

Batch experiments were conducted to measure:

- Zn, Cu, Pb and zeolite adsorption in a mixed singly metals – zeolite – *Bacillus* system
- Zn, Cu, Pb and bentonite adsorption in a mixed singly metals – bentonite – *Bacillus* system second
- Zn, Cu, Pb and quartz sands adsorption in a mixed singly metals – quartz sands – *Bacillus* system
- Zn, Cu, Pb and calcite adsorption in a mixed singly metals – calcite – *Bacillus* system
- Zn, Cu, Pb and magnezite adsorption in a mixed singly metals – magnezite – *Bacillus* system

Z e o l i t e

The natural materials, zeolite was obtained from Nižný Hrabovec location in Slovakia. The mineralogical composition of zeolite was clinoptilolite 51–68 %, quartz + cristobalite 9–20 %, feldspars 8–13 %, mica 13 % and iron minerals 0.3 %.

Table I
Chemical composition of zeolite

Components	SiO_2	Al_2O_3	Fe_2O_3	MgO	Na_2O
% wt.	67.0	12.3	1.3	0.7	0.7

B e n t o n i t e

The natural materials, zeolite was obtained from Lastovce location in Slovakia. The mineralogical composition of bentonite was smectite 63%, quartz 21%, kaolinite 11%, feldspars 4-6% and calcite 2%.

Table II
Chemical composition of bentonite

Components	SiO_2	Al_2O_3	Fe_2O_3	MgO	Na_2O
% wt.	59.2	18.6	2.8	4.2	0.7

Q u a r t z S a n d s

The natural materials, zeolite was obtained from Nižný Hrabovec location in Slovakia. The mineralogical composi

tion of zeolite was quartz 85 %, kaolinite 8 %, mica 6 % and iron minerals 3–5 %.

Table III
Chemical composition of quartz sands

Components	SiO_2	Al_2O_3	Fe_2O_3	MgO	Na_2O
% wt.	96.1	1.7	0.4	0.05	0.03

C a l c i t e

The natural materials, calcite was obtained from Horné Srnie location in Slovakia. The mineralogical composition of rock was calcite 80 %, quartz 5 %, kaolinite 2 %, plagioclase 0.5 %, and iron minerals 0.7 %.

Table IV
Chemical composition of calcite

Components	SiO_2	Al_2O_3	Fe_2O_3	MgO	Na_2O
% wt.	11.8	3.9	1.2	0.8	0.05

M a g n e z i t e

The fired material of magnezite was obtained from Jelšava location in Slovakia. The mineralogical composition of sample was periclase 98 %.

Table V
Chemical composition of fired magnezite

Components	SiO_2	CaO	Fe_2O_3	MgO	K_2O
% wt.	0.02	0.11	0.01	97.3	0.06

Results

The individual mineral phases of silicates (zeolite, bentonite, quartz sands) and carbonates (magnezite, calcite) can control the efficiency of biofiltration when sorption and precipitation of metals occurs. Moreover, in both cases, Zn, Cu, Pb were accumulated at the mineral and cell surface as precipitates. An important parameter for packing material is the removal capacity of the minerals.

Removal of Zn from solution by silicate was of lower efficiency, 20 % Zn by zeolite, 46 % by bentonite, 21 % by quartz sands was removed, while up to 55 % by calcite and 88 % by magnezite (Fig. 1.).

Removal of Cu was similar approximately 24 % by these minerals (Fig. 2.).

Pb was very effective removed 93 % by bentonite and magnezite and 74 % by zeolite and calcite (Fig. 3.).

The experimental results of the selected natural mineral materials showed that bentonite has higher adsorption capacities than zeolite for Pb and Zn. However, magnezite appear to be more effective than calcite in precipitation of Pb and Zn.

The affinity series for bacterial removal of these metals decrease in the order Pb > Zn > Cu with the low sorption capacity only approximately 0.06 mM dm^{-3}. At 0.1 g bacteria dm^{-3} is a small amount of cells for adsorption metals onto the bacterial surface.

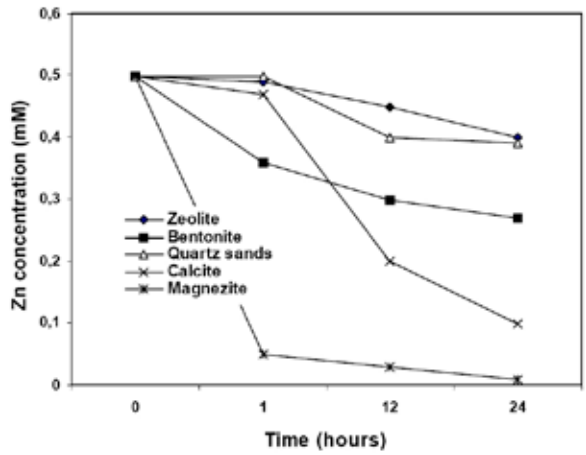

Fig. 1. **Time dependence of Zn biosorption and precipitation by minerals and bacterial cells**

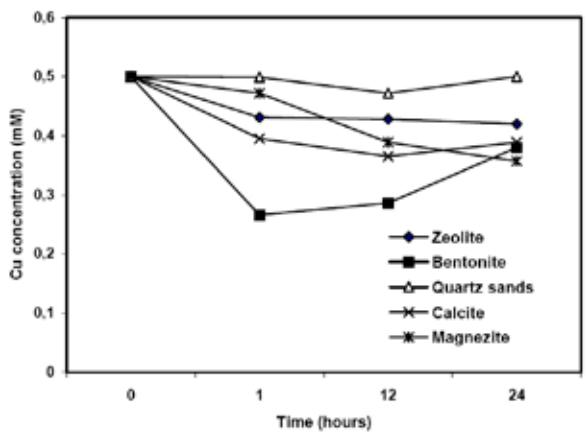

Fig. 2. **Time dependence of Cu biosorption and precipitation by minerals and bacterial cells**

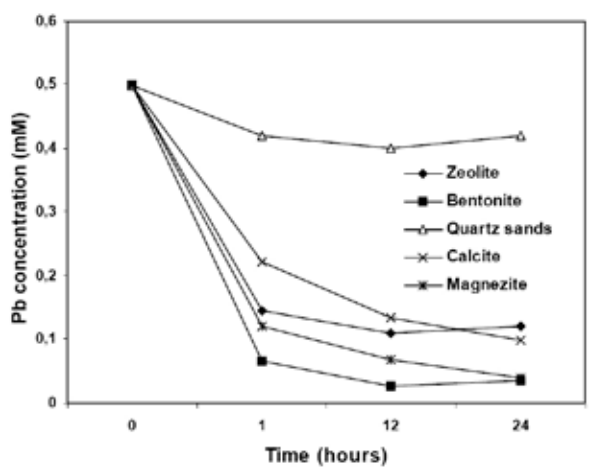

Fig. 3. **Time dependence of Pb biosorption and precipitation by minerals and bacterial cells**

This biosorption batch experiment represents a minerals-dominated regime in which the bacterial concentration is low, and therefore the difference of mineral and cell adsorption is big in the biological processes of pH change from 7 to 4 during silicate biofiltration and of pH change from 7 to 9 during carbonate biofiltration.

The result dominantly involves decreasing metal concentration in solution in dependence of time and kind of minerals. This indicates that the contact time between the metal solution and the mineral kind and also the bacterial amount is of crucial importance.

Conclusions

The batch adsorption experiment in ternary and binary systems with individual sorbents, ternary metal-mineral-bacteria and binary metal-bacteria interaction can be used to ascertain the effect of bacteria and selected of minerals like packing materials in development of biofilters. The results suggest that the adsorption observed in the 0.1 g dm^{-3} bacterial dm^{-3} system dominantly involves the mineral surface that is why biofiltration need to increase bacterial amount in packing filter.

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P71 ANTIBIOTIC EFFECTS OF THE NAPHTOQUINONIC DERIVATIVE ON GRAM-POSITIVE AND GRAM-NEGATIVE GERMS

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Introduction

Intestinal motile bacteria *Enterobacter aerogenes* and nonmotile coccus *Enterococcus faecalis* are known as being contaminating pathogenetic agents of aliments[1–3] and drinking water[4]. Pathological agents: coccus and bacteria have a significant multiple-antibiotic resistance at natural drugs and also at chemically obtained drugs.[5–10]

The naphthoquinones are a wide class of plants' metabolites, which are currently used for manufacturing cosmetics, foods or for medicinal purposes[11,12]. Also the natural naphthoquinones were tested for antitumoral[13], antiinflamatory[14] and antimicrobial[15,16] activity. For antibiotic activity is being responsible the compound structure, therefore the aim of any structure-activity relationship should be to find the most potent and least toxic compound. In this respect, we studied the antibiotic effect of dichlor derivative of 1,4-naphthoquinone: 2,3-dichloro-1,4 naphthoquinone (dichlone – Fig. 1.) on Gram-positive nonmotile coccus *Enterococcus faecalis*, and Gram-negative motile bacteria *Enterobacter aerogenes*.

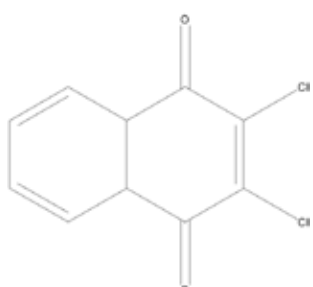

Fig. 1. **Dichlone structure**

In order to identify dichlone's grade of remanence, in the inoculated culture mediums, it was used HPLC technique.

Experimental

M a t e r i a l s a n d E q u i p m e n t s

Dehydrated culture media: primary culture media TSA (tryptic soy agar) from Fluka (Buchs, Switzerland) and M-H broth (Müeller-Hinton broth) from Fluka (Buchs, Switzerland) for antibiotic susceptibility testing.

Both culture media were rehydrated with ultrapure water made with water ultra-purifier TKA Smart2Pure UV6 from TKA Wasseraufbereitungssysteme GmbH (Niederelbert, Germany).

Used microbial strains were made by MicroBioLogics Inc. (Saint Cloud, USA), as following:

- KWIK-STIK™: *Enterococcus faecalis* (ATCC 29212);
- KWIK-STIK™: *Enterococcus faecalis* (ATCC 19433);
- KWIK-STIK™: *Enterobacter aerogenes* (ATCC 13048).

Culture media were sterilised with a Raypa AES-75 autoclave from R.ESPINAR, S.L. (Barcelona, Spain).

All cultures were incubated in a Raypa Incuterm ID-50 incubator from R.ESPINAR, S.L. (Barcelona, Spain).

Barium chloride from Utchim S.R.L. (Râmnicu Vâlcea, Romania) and sulfuric acid from Utchim S.R.L. (Râmnicu Vâlcea, Romania) were used for preparation of McFarland turbidity standards.

Odyssey DR/2500 spectrophotometer from Hach Company (Loveland, USA) was used for inoculums' and prepared McFarland turbidity standards' OD (optical density) determination.

For antimicrobial susceptibility testing it was used dichlone from Merck (Darmstadt, Germany).

Dichlone's grade of remanence was detected with HPLC equipment: Thermo Finnigan Surveyor system from Thermo Fisher Scientific Inc. (Waltham, USA). PDA detector set to 254 nm. Hypersil™ GOLD Column 100 × 4.6 mm.

Dichlone standard for HPLC: dichlone standard from Supelco (Bellefonte, USA).

Other reagents for HPLC technique:

- Acetonitrile from Merck (Darmstadt, Germany);
- Water Chromasolv from Honeywell International Inc., Riedel-de Haën (Seelze, Germany).

M e t h o d s

Primary and secondary cultures: the KWIK-STIK™ lyophilized microorganisms' strains (*Enterococcus faecalis* ATCC 29212 and ATCC 19433; *Enterobacter aerogenes* ATCC 13048) were transferred on Petri dishes with sterile TSA and incubated at 35 °C for 24 hours (primary cultures); then, subcultures were made from primary cultures and incubated also on sterile TSA for 24 hours at 35 °C.

Antimicrobial susceptibility testing by broth microdilution technique: dichlone's MIC (minimal inhibitory concentration – defined as drug concentration at which no growth is visible) was determined by broth microdilution technique. A sterile culture tube with M-H broth was inoculated with an aliquot from the three types of secondary growth. Inoculated broth's OD was adjusted at 0.5 McFarland standard with prepared turbidity standards – aproximatively 1×10^8 CFU ml^{-1}. Standardised inoculums' aliquots from all three types of secondary culture were transferred on culture tubes with sterile liquid medium: M-H broth. Dichlone's MIC was tested on those standardised inoculums, on M-H broth.

Table I
Concentration of dichlone used in MIC testing

Dilution 1	Dilutions 2	Dilutions 3
0.001 μg ml^{-1}	0.125 μg ml^{-1}	16.0 μg ml^{-1}
0.002 μg ml^{-1}	0.25 μg ml^{-1}	32.0 μg ml^{-1}
0.004 μg ml^{-1}	0.5 μg ml^{-1}	64.0 μg ml^{-1}
0.008 μg ml^{-1}	1.0 μg ml^{-1}	128.0 μg ml^{-1}
0.016 μg ml^{-1}	2.0 μg ml^{-1}	256.0 μg ml^{-1}
0.032 μg ml^{-1}	4.0 μg ml^{-1}	
0.064 μg ml^{-1}	8.0 μg ml^{-1}	

HPLC technique: for the identification of 2,3-dichloronaphtoquinone in the samples a 1 mg ml^{-1} solution was prepared in acetonitrile and 10 μl injected into the HPLC system. The sample was injected three times.

Mobile phase was 60 % acetonitrile with 40 % water.

Chromatograms were registered at 254 nm.

Results

Antimicrobial susceptibility testing results show that the dichlone have no antimicrobial activity on Gram-negative bacteria *Enterobacter aerogenes*; in the same time the growth of Gram-positive coccus *Enterococcus faecalis* (both strains) was completly inhibited, as in Table II.

Table II
Dichlone's MIC on Enterococcus faecalis strains

Strain	MIC [μg ml^{-1}]
E. faecalis – ATCC 29212	0.5
E. faecalis – ATCC 19433	0.5

As we mentioned in introduction, dichlone's grade of remanence, in the inoculated culture mediums for MIC was determined by HPLC. The chromatograms of the mentioned naphtoquinone obtained at the most effective chromatographic conditions are shown in Figs. 2. and 3.

The height and area of PDA signal (254 nm) for this naphtoquinone confirmed the calculated value for MIC.

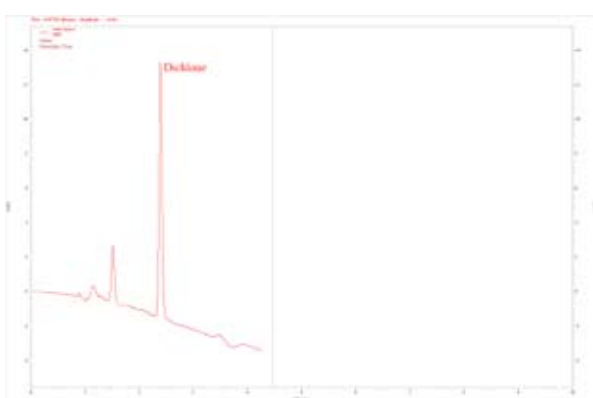

Fig. 2. **HPLC chromatogram for dichlone's MIC (*E. faecalis* – ATCC 29212)**

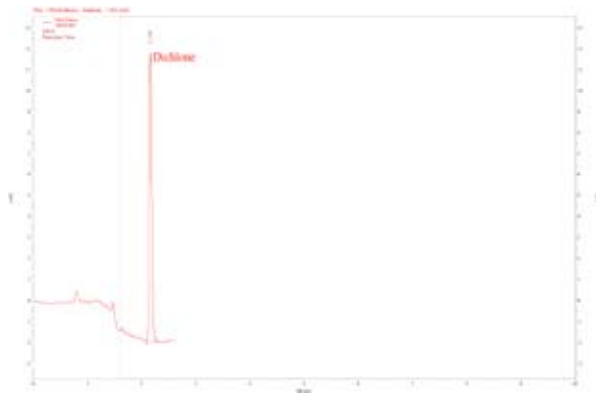

Fig. 3. **HPLC chromatogram for dichlone's MIC (E. faecalis – ATCC 19433)**

Conclusions

The results of this study show that dichlone has no antimicrobial activity on Gram-negative germs (*Enterobacter aerogenes*). In the mean time, dichlone's MIC on Gram-positive germs (*Enterococcus faecalis* – both strains) it was at 0.5 μg ml^{-1} in liquid culture media (M-H broth). This concentration was confirmed by HPLC determination.

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P72 PRACTICAL APPLICATION IN AGRICULTURE OF THE MAGNESIUM PRODUCTS INDUSTRY WASTE

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Introduction

The industrial processes of manufacturing magnesium compounds – oxide and carbonates mainly, from dolomites, by carbon dioxide leaching, generate important amounts of waste[1,2]. The composition of this waste includes calcium carbonate and precipitated magnesium carbonates (in ratio of 3:1 till 4:1) together with other impurities, presents in the raw material such as iron, manganese, copper and zinc[3,4].

The alkaline reaction and the important mineral content – essential and trace elements – of this waste can be valuated in agriculture as soil amendment and fertilizer for acid soils with low fertility[5–7].

The main objective of this study is to present the influence of waste types and doses on the fertility characteristics of an acid soil. The improve of the soil fertility was established by studying some vegetation characteristics and the protein content of green oat plants. The paper reports the effects of several waste doses and types on luvosoil with and without nitrogen contribution. Two types of waste were experimented, one from the industrial process (A) and the second resulted as crusts deposed on the equipment walls (B).

Experimental Part

Luvosoil, having a pH_{H2O} of 6.94 and pH_{KCl} of 5.76 and a rather low soil fertility, was collected, air-dried, crushed, mixed and put into pots, each containing 1 kilogram soil. The soil was treated with two types of waste in different amounts, having each the composition presented in Table I.

Table I
Composition of the two experimented industrial waste

Specification	Waste A	Crusts B
Ca, %	28	19
Mg, %	7	14
Fe, mg kg^{-1}	1850	880
Cu, mg kg^{-1}	1.9	51
Mn, mg kg^{-1}	136	51
Zn, mg kg^{-1}	2.6	50

The experimental alternatives pursued by this research consist of four different doses for each waste (A, B), namely A_1, A_2, A_3, A_4 and B_1, B_2, B_3, B_4 and also a control alternative (C_0), represented by untreated soil. All the experimental alternatives took place in three replicates (R_1, R_2, R_3). At the replicates R_2 and R_3, 134 mg nitrogen kg^{-1} soil as ammonium nitrate was added in each pot. The description of the experimental alternatives is shown in Table II, in which R represent the replicate without nitrogen treatment (R_1) and R_N – the average of replicates R_2 and R_3, treated with nitrogen.

All the pots were sown with thirty oat grains. The vegetation period was that of green plant, pursued for 8 weeks. The pots were placed in laboratory near the window and watered every second day by 100 ml water. Along the vegetation period, some morphological parameters, like number of risen plants, plant size, fresh and dry weight were pursued. Some composition features like dry matter and protein content were determined too. At harvest time, soil samples were collected in order to establish the impact of waste treatment on soil fertility. Soil characteristics like pH, essential and trace elements were analysed.

Soil pH in watery and salin extracts was determined by a pH-meter. The metal element content in soil at harvest time was established by AAS-ICP method. The protein content in oat plants was analysed by using the Kjeldahl method.

Results and Discussion

The impact of waste treatment on soil reaction and macroelements content is shown in Table III.

Soil reaction was analysed using two analytical methods, in watery and salt extract. The extraction in KCl solution hinders salts hydrolysis in soil and therefore the obtained pH values are more stable but lower by 1.7 pH units in comparison with those of the watery extract. For both methods, a buffer process of the soil reaction was established, turning the low acid soil reaction to an alkaline reaction, proportional with the increase of the waste dose. The increase of the pH value took place slowly by adding waste A and suddenly in case of waste B, which composition contains more magnesium than that of waste A. The highest pH values were established for A_4 (pH = 8.354) and B_4 (pH = 8.290). The nitrogen contribution decreases the pH-values because of the acid reaction of ammonium nitrate.

Analysing the soil calcium content, a proportional increase was established once with the growth of the waste dose. The increase of the calcium content took place suddenly for waste of type A and slowly for the waste B, remarking a suddenly growth only for the highest B dose. The highest waste doses for type A and B generate a calcium content of 2.674 g kg^{-1} (A_4) and 2.717 g kg^{-1} (B_4) respectively.

A growth of the magnesium soil content was established once with the increase of the waste dose (A, B). Higher values were determined for waste type B. Alike as for the calcium content in soil, a nitrogen contribution decreases the magnesium content values for the alternatives with waste of type A.

Table II
Description of the experimental alternatives

Experimental alternative		Waste dose, mg kg^{-1}	Nitrogen contrib., mg kg^{-1}	Mineral Suplimentation Ca, mg kg^{-1}	Mg, mg kg^{-1}	Fe, mg kg^{-1}	Cu, µg kg^{-1}	Mn, µg kg^{-1}	Zn, µg kg^{-1}
A_1	R	179	-	50	13	0.33	0.34	24.3	0.47
	RN	179	134	50	13	0.33	0.34	24.3	0.47
A_2	R	357	-	100	25	0.66	0.68	48.7	0.93
	RN	357	134	100	25	0.66	0.68	48.7	0.93
A_3	R	714	-	200	50	1.32	1.36	97.4	1.86
	RN	714	134	200	50	1.32	1.36	97.4	1.86
A_4	R	1429	-	400	100	2.64	2.72	194.7	3.72
	RN	1429	134	400	100	2.64	2.72	194.7	3.72
B_1	R	263	-	50	37	0.23	13.40	13.4	13.20
	RN	263	134	50	37	0.23	13.40	13.4	13.20
B_2	R	526	-	100	74	0.46	26.80	26.8	26.40
	RN	526	134	100	74	0.46	26.80	26.8	26.40
B_3	R	1053	-	200	147	0.93	53.60	53.6	52.60
	RN	1053	134	200	147	0.93	53.60	53.6	52.60
B_4	R	2105	-	400	295	1.85	107.30	107.3	105.20
	RN	2105	134	400	295	1.85	107.30	107.3	105.20

Table III
Impact of waste treatment on soil reaction and macroelements content

Experimental alternative		Soil reaction pH_{H2O}		pH_{KCl},		Ca content		Mg content	
		pH unit	D	pH unit	D	g kg^{-1}	%	g kg^{-1}	%
C_0	R	6.939	-	5.763	-	2.136	100.0	0.327	100.0
A_1	R	6.887	–0.052	6.093	0.330	2.267	106.1	0.348	106.4
	RN	7.054	0.115	5.934	0.171	2.222	104.0	0.343	104.9
A_2	R	7.718	0.779	6.565	0.802	2.261	105.8	0.352	107.6
	RN	7.229	0.290	6.174	0.411	2.168	101.5	0.337	103.1
A_3	R	8.178	1.239	6.468	0.705	2.440	114.2	0.400	122.3
	RN	8.291	1.352	6.983	1.220	2.280	106.7	0.371	113.5
A_4	R	8.354	1.415	7.307	1.544	2.674	125.2	0.464	142.2
	RN	8.435	1.496	7.257	1.494	2.607	122.0	0.449	137.3
B_1	R	7.771	0.832	6.216	0.453	2.122	99.3	0.343	104.9
	RN	7.386	0.447	5.908	0.145	2.151	100.7	0.330	100.9
B_2	R	7.734	0.795	6.556	0.793	2.164	101.3	0.361	110.4
	RN	7.677	0.738	6.536	0.773	2.270	106.3	0.380	116.2
B_3	R	8.016	1.077	6.881	1.118	2.254	105.5	0.412	126.0
	RN	8.215	1.276	6.944	1.181	2.390	111.9	0.427	130.6
B_4	R	8.290	1.351	7.082	1.319	2.717	127.2	0.566	173.1
	RN	8.222	1.281	7.360	1.597	2.547	119.2	0.480	146.8

Table IV
Influence of waste type and doses on the soil trace elements content

Experimental alternative		Fe content		Mn content		Zn content		Cu content	
		$\mu g\,kg^{-1}$	%	$\mu g\,kg^{-1}$	%	$g\,kg^{-1}$	%	$g\,kg^{-1}$	%
C_0	R	234.7	100.0	87.84	100	11.400	100.0	3.402	100.0
A_1	R	249.6	106.3	84.20	95.9	33.100	290.4	3.491	102.6
	RN	251.4	107.1	84.30	96.0	25.080	220.0	3.639	107.0
A_2	R	244.6	104.2	80.06	91.1	31.200	273.7	3.669	107.8
	RN	245.1	104.4	95.00	108.2	11.900	104.4	3.669	107.8
A_3	R	260.3	110.9	86.97	99.0	13.960	122.5	3.462	101.8
	RN	253.6	108.1	85.44	97.3	11.910	104.5	3.609	106.1
A_4	R	257.0	109.5	73.42	83.6	12.920	113.3	3.669	107.8
	RN	264.5	112.7	88.00	100.1	6.721	59.0	3.821	112.3
B_1	R	232.5	99.1	75.56	86.0	4.418	38.8	3.462	101.8
	RN	251.1	107.0	95.77	109.0	4.407	38.7	3.609	106.1
B_2	R	242.5	103.3	70.53	80.3	4.832	42.3	3.358	98.7
	RN	236.6	100.8	81.62	92.9	4.821	42.3	3.462	101.8
B_3	R	235.0	100.1	94.39	107.5	4.567	40.1	3.787	111.3
	RN	237.8	101.3	73.76	84.0	19.300	169.3	3.506	103.1
B_4	R	253.8	108.1	92.62	105.4	4.425	38.8	3.728	109.6
	RN	237.8	101.3	76.49	87.1	10.210	89.6	3.402	100.0

Table V
Influence of waste and nitrogen contribution on some vegetation characteristics of green oat

Experimental alternative		Risen plants		Size of green plants		Fresh weight	
		number	%	cm	%	$mg\,piece^{-1}$	%
C_0	R	21	70	51	100	362	100
A_1	R	22	73	54	106	327	90
	RN	23	77	73	143	591	163
A_2	R	27	90	53	104	352	97
	RN	22	73	72	141	623	172
A_3	R	28	93	56	109	332	92
	RN	22	73	75	147	650	180
A_4	R	23	77	59	116	387	107
	RN	19	63	77	151	737	204
B_1	R	22	73	53	104	300	83
	RN	27	90	70	137	537	148
B_2	R	24	80	60	118	350	97
	RN	22	73	78	153	600	166
B_3	R	25	83	52	102	404	112
	RN	28	93	70	137	464	128
B_4	R	30	100	53	104	370	102
	RN	22	73	58	114	582	161

Table VI
Impact of waste treatment on some composition features of green oat plants

Experimental alternative		Dry weight		Dry matter	Protein content	
		mg piece^{-1}	%	D.M. %	P%	%
C_0	R	162	100	44.8	10.08	100
A_1	R	159	98	48.1	5.42	53.8
	RN	248	153	41.9	12.56	124.6
A_2	R	167	103	46.9	4.75	47.1
	RN	255	157	41.0	12.65	125.5
A_3	R	161	99	47.9	5.01	49.7
	RN	255	157	39.2	13.68	135.7
A_4	R	183	113	47.3	5.03	49.9
	RN	300	185	41.0	12.78	126.8
B_1	R	155	96	51.3	5.41	53.6
	RN	233	144	43.0	12.31	122.1
B_2	R	158	98	45.6	4.98	49.4
	RN	273	169	46.0	11.89	118.0
B_3	R	184	114	46.5	4.63	45.9
	RN	200	123	43.4	12.59	124.9
B_4	R	150	93	40.8	5.83	57.8
	RN	250	154	42.9	12.99	128.9

Because of their trace elements content, the soil treatment with waste of type A and B generates in soil a different trace element level in comparison to the control alternative, presented in Table IV.

Analysing the iron content of the experimental alternatives, it was established that unimportant increases of the iron content took place once with the growth of both waste type doses. The highest iron concentrations were found in alternative A_4 and B_4 representing the highest waste doses and having an increase of 12.7 % (A) and 8.1 % (B).

The manganese content in soil has lower values for all the experimental alternatives than that of the control alternative. An exception is represented by A_2 registering an increase of 8.2 % and B_1 of 9.0 %, both with nitrogen contribution.

The zinc content in soil decreases once with the increase of the waste dose. The highest value was registered for A_1 and the increase was of 190.4 %. By addition of waste B, the zinc content in soil decreases having lower values than the control alternative.

The copper content in soil remains almost constant after waste addition. The highest increase was registered for A_4 representing 12.3 %. Treating soil with waste B, the highest registered increase was 11.3 % for B_3.

The effects of soil treatment with waste A and B show an important influence on the development and nutrition of green oat plants. The results are presented in Table V and Table VI.

The enhance of the waste A amounts in soil treatment had a beneficial effect on the grain germination praised by a higher number of risen plants. Adding nitrogen to soil, the number of risen plants remained low. The effects are similar for soil treatment with waste B, except for B_1 and B_3, where the nitrogen contribution increases the number of risen plants. The highest number of risen plants was established for B_4, 30 representing 100 % of the sown oat grains.

Green oat plants grew taller once with the increase of the waste dose in both cases (waste A and waste B). Adding nitrogen, an obvious increase of the plant size was established. The tallest plants were find for alternatives A_4 (highest waste A dose + nitrogen contribution), namely 77 cm and 78 cm for B_2 (second waste B dose + nitrogen contribution).

At harvest time, green oat plants for all alternatives were thinner, having a reduced fresh weight in comparison with the control alternative for those without nitrogen contribution. The nitrogen supplement makes the plants more vigorous having a higher fresh weight and dry weight for all the experimental alternatives. The fresh weight increase was more evident for soil treatment with waste A; for the highest waste dose (A_4), the increase was of 104 % in comparison with the control alternative. Similar to the fresh weight increase, took place the dry weight increase. The most evident results were established for the alternatives treated with waste A. The increase of the dry weight was the highest of 85 % for the alternative A_4.

The altering of dry matter was increasing for the alternatives without nitrogen treatment and decreasing for those with nitrogen treatment. The highest dry matter value was of 51.3 % for B_1 (lowest waste B dose) and the lowest value was 39.2 % for A_3 (waste A + nitrogen contribution).

The protein content of green oat plants shows, at harvest time, two different aspects comparative with the control alternative. For the alternatives in which no nitrogen was added, the protein content represents half of the control alternative content. Adding nitrogen, the protein content has increased and became double given to the alternatives without nitrogen contribution. The highest protein level was found for

A_3, namely 13.68 % and 12.99 % for B_4. The increase of the waste dose and nitrogen contribution generates an enhance of the plant protein content.

Conclusions

Considering the obtained results, the two experimented industrial waste can be used in certain doses as soil amendment for low fertile acid soil, with or without nitrogen addition.

Treating soil with different doses of waste, a buffering effect was established, which rises the pH values from low acid to low alkaline.

The presence of magnesium and calcium in the waste composition increases the soil content, proportional with the growth of the waste dose.

The enhance of the trace elements content in soil is representative for iron, copper and zinc (waste A).

The experimental waste doses (waste A, waste B) had a beneficial effect on grain germination improving the number of risen oat plants by 23 % (waste A) and 30 % (waste B).

At harvest time, the size of green oat plants was taller by 8 cm (waste A) and 9 cm (waste B) comparative to the control alternative with untreated soil. Nitrogen additions increased their size by 51 % (waste A) and by 53 % (waste B).

The fresh weights of the plants show a decrease proportional with the increase of the waste doses (A, B) only for the alternatives without nitrogen addition.

The dry matter values are increasing once with enhance of dose for both waste in all alternatives without nitrogen contribution. The addition of nitrogen decreases the dry matter value at harvest time for all the alternatives comparative to the control. The decrease of dry matter is more severe for the alternatives treated with waste A.

Adding nitrogen, the protein content has increased and became double given to the alternatives without nitrogen contribution.

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P73 POSSIBILITY OF OBJECTIVE CONTROL OF NATURAL GAS ODORISATION

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Introduction

Natural gas is one of the most important energy carriers in Europe (in the Czech Republic as well). Total consumption of natural gas (NG) in the Czech Republic in 2006 was $9{,}269 \times 10^6$ m^3. Within the distribution to end users and also during natural gas utilization the most important requirement is the safety of customers.

Natural gas odorisation means operations involving the addition of odorant to gas to ensure characteristic odour of NG so that a person can judge the odour to be distinctive and unpleasant so that the presence of gas in air (in concentrations below lower explosive limit – LEL) is readily detectable. By the odorant addition any physical or chemical property (except the smell) of NG cannot be changed. As odorants organic sulfur compounds are often use (mercaptans and sulfides). Nowadays a new type of sulfur free odorant is being introduced to the NG market.

The NG odorisation in fact does not have any technological purpose; its main sense consist in evoking psychological effect, because the odour of NG must be alarming and incommutable with any common smell.

In the Czech Republic the odorisation process is specified by the technical regulation TPG 918 01. This regulation lists as odorants just organic sulfur compounds. However, it does not mean that sulfur-free odorants can not be used.

Experimental

The main task of natural gas odorisation is to ensure such operating condition when natural gas in every part of the distribution grid fulfils the requirement of a "warning odour level". In case of a gas leakage the warning odour level (see Table I.) must be reached until the 20 % of lower explosive limit (LEL; L_d) is reached. Odorisation level can be verified by:

- The odorisation level control – which can be done by olfactometry in selected points on distribution grid or by means of questionnaires at selected representative sample of customers. In both cases indirect indicators are taken into account so that both forms are considered to be subjective methods.
- Odorant concentration measurement – in natural gas can be estimated continuously or discontinuously in selected points on grid. In this case particular concentration of odorant in NG is measured. This is so called objective method.

The aim of this work was a critical comparison of the subjective odorisation control (according to TPG 91801) with the objective odorisation control. It means direct measurement of an odorant concentration in NG using modern analytical techniques and comparison with results from olfactometry measurement.

Analytical Equipment

The accurate odorant concentration in NG was estimated by gas chromatograph HP 6890 equipped with mass detector MSD 7393 (Hewlett-Packard). For the analysis Supelco 24158 SPB-1 Sulfur (30 m × 320 μm × 4 μm) column was used. Starting temperature was 30 °C with heating rate 15 °C min^{-1}. The analysis terminated at 110 °C.

The odorisation level control was estimated by dynamic olfactometry using olfactometer Ecoma TO 8-8. The procedure is based on step-by-step evaluation of olfactory perception of at least four (max. eight) observers. A sample of odorised natural gas is diluted by synthetic air in ratios between 1 : 131,072 and 1 : 8 (NG:air). As soon as the observer indicates olfactory perception to be odour treshold (or warning odour level) the actual dilution of the sample is recorded.

Terminology

Minimal odorant concentration represents the odorant content in NG [mg m^{-3}] which fulfill the requirement for creating warning odour level – grade 3 (see Table I).

Estimation of the minimal odorant concentration is determined by:

- K value [mg m^{-3}] which represents the minimal concentration of an odorant in natural gas-air mixture which reliably ensures the warning odour level,
- lower explosive limit (L_d) –expressed by % vol. of natural gas in air,
- and from the requirement to evoke the warning odour level before one fifth (i.e. 20 %) of LEL of natural gas in air is reached.

Minimal odorant concentration c_n can estimated according to the following formula:

$$c_n = \frac{100 \cdot K}{0.2 \cdot L_d} \ [\mathrm{mg\,m^{-3}}]. \qquad (1)$$

Typical K values of commonly used odorants are 0.08 for tetrahydrothiophene, 0.03 for mercaptans and 0.07 mg m^{-3} for the GASODOR S-free odorant.

Odour intensity is the extent of odour perception which is by the odour evoked. Commonly the odour intensity is evaluated as an odorisation level. List of odorisation levels can be found in the Table I.

Results

Two samples of a real odorised "Russian" natural gas sampled directly from natural gas pipeline into tedlar sam-

ple bags were used for all experiments. Both samples were analyzed by GC-MS and an overview of obtained odorant concentrations is given in Table II.

Table I
Odorisation Levels according to TPG 918 01(ref.[2])

Odorisation level (grade)	Olfactory perception	Comment
0	Odour not detected	–
1	Very low intensity	Odour threshold
2	Weak odour	–
3	Mean odour	Warning odour level
4	Strong odour	–
5	Very strong odour	–
6	Extremely strong odour	Upper limit of intensity

Table II
Samples overview

	DMS	TBM [mg m^{-3}]	Total
Sample 1	2.71	3.31	6.02
Sample 2	3.32	4.35	7.67

For the estimation of odorisation level shortened examination described in technical norm ČSN 38 5550[1] was applied. In this test the odour threshold (grade 1) is estimated as a first point and the warning odour level (grade 3) as a second point. From obtained data (mean value from all observers mean values) the dependence of odorisation level on odorant concentration in natural gas was created.

The measurement was carried out by two different groups of observers.

The first one (GROUP 1), consisted of professional observers who satisfied the conditions listed in ČSN EN 13725[3] for performing the olfactometry measurement.

The second one (GROUP 2), consisted of observers who are performing the subjective odorisation control in gas distribution companies.

Obtained data are given in Figs. 1. and 2. Each of them represents the dependence of odorisation level on the odorant concentration in natural gas – air mixture.

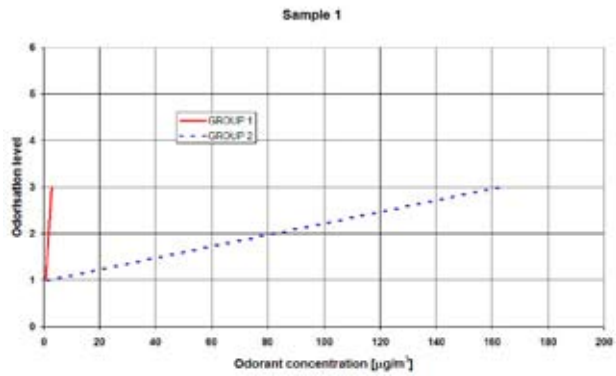

Fig. 1. **Dependence of odorisation level on the odorant concentration for Sample 1**

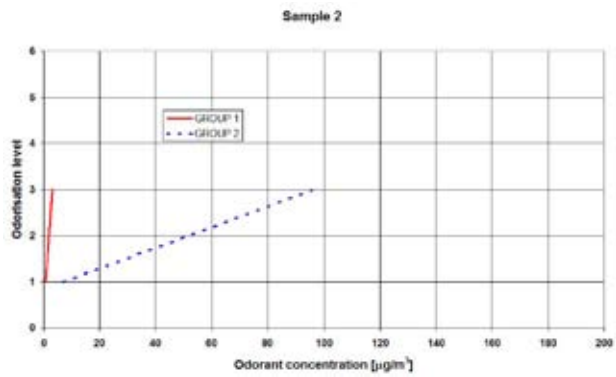

Fig. 2. **Dependence of odorisation level on the odorant concentration for Sample 2**

Conclusions

Performed measurement shows considerable subjectivity of an olfactometry measurement of the odorisation level. While observers from Group 1 respond accurately with minimal deviations, observers from Group 2 respond in wide range of dilution with considerably scattered results. This result is connected with the sensitivity threshold across population and to some extent with professional deformation of employees carrying out the on-site olfactometry odorisation control of NG at selected points on natural gas grid.

From the data obtained from Group 1, the *K* value for the odorant used in samples can be estimated. This value is considerably lower than the *K* value, which is currently used in the Czech Republic (the obtained *K* value lies under 0.003 mg m^{-3} comparing to published 0.07 mg m^{-3} in the national technical regulation[2]).

As a main result of accomplished experiments it can be strictly recommended to prefer objective method for periodic odorisation control. Nowadays gas distribution companies slowly change the way of odorisation control considering the objective control as the guarantee of safe gas distribution and utilization up to end customers.

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P74 CONTAMINATION OF SOIL AND ALIMENTARY WHEAT IN ZEMPLÍNSKA POLLUTED AREA

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Introduction

Soil is a determining component of environment by its range and function that belongs to the basic technologic instruments and it is not an only revealing resource. Agrocosystems and mainly soil of these actively react on entries of polluted substances, which cause its biological degradation. Inorganic contaminants belong to main factors of biological degradation, heavy metals and metalloids are cummulated in soil environment. Our soils situated in key area (Strednozemplínska area) are exposed to an emissions effect from many sources for a long time period. It is proved mainly by acidification of soil horizons, exceeded content of heavy metals in soil and consequently in above-ground mass in this contaminated were found.

Experimental

We have collected the soil samples from exactly given places by the GPS system. Then we have processed information in the OziExplorer program, where the gained points were also evaluated. We have gained the soil samples from two depths 0–0.1 m (A horizon) 0.2–0.35 (B horizon). The soil samples have been taken by geological probe GeoSampler. The contents of the risky elements has been elevated on areas with active agricultural activity in Humenné location where we could assume that the observed area is contaminated by the reason of widespread industrial activity in this region. The contents of risky elements were determinated in *aqua regia* as a pseudototal content in 2M HNO_3 as the potential mobilized forms.

Results

The total content of the risky elements in soils includes all forms of the occurrence of exact element in soil. In our soil samples we have determined the contents of these chosen risky elements Cd, Pb, Ni, Zn, Cu, Cr, Co. We have evaluated the measured results by law No. 220/2004 about protection and exploitation of agricultural soil and resolution No. 531/1994. The evaluated area has exceeded the limit values in *aqua regia* solution in A horizon in the case of four following risky elements Cd, Ni, Zn and Co. Content of Cd was increased in all gained points in the observed horizon. The interval of values has been in range of 1.10 to 1.90 mg Cd kg^{-1} soil. The highest value presented was exceeding 171 % to the limit value. The content of Ni moved in wide interval of 40.4–70.4 mg Ni kg^{-1} of soil. At the highest content the limit value has exceeded by 40.8 %. The limit value of Zn enhanced in one gained point where the value presented 153.2 mg kg^{-1} that means exceeding at 2.1 %. The content of Co moved in wide interval from 19.6–29.6 mg kg^{-1}. The limit value for Co exceeded in all gained points. Limit value was at the highest concentration enhanced by 97.3 %.

In B horizon in digestion of *aqua regia* the limit values of all metals also exceeded. The content of Cd was increased in the observed horizon by all gained points. The interval of the value moved from 1.30 to 2.0 mg Cd kg^{-1} of soil. The content of Pb moved in wider range 39.6–114.8 mg Pb kg^{-1} of the soil and it was measured in two gained points. The highest content means increase at 64 %. The content of Ni exceeded in six gained points. The highest content presented the value 52.8 %. The increased content of Cu and Cr at the present area has a detailed character; the highest value of Cu exceeded the limit value at 12.6 %. The highest measured value of Cr, 86.8 mg kg^{-1} soil means increasing by 24 %. Measured values of Co we have found were in a range from 19.2–40.0 mg kg^{-1}. The limit value for Co was exceeded in all gained points. The content of heavy metals in 2M HNO_3 solution represented the potential mobilized content which includes different fraction of elements from the view of their solubility. The content of Cd in A horizon in Humenné area has moved in the range of interval 1.9–3.2 mg kg^{-1}. The value 3.2 mg kg^{-1} presented 10.6 fold increasing towards the reference value. The contamination by Cd has a broad character and affects all the gained points. The measured value of Pb was in the range 13.1–185.0 mg kg^{-1}. Other values of the heavy metals which were measured in A horizon doesn't express the enhanced potential mobility and their contents were under the limit value. The content of Cd in B horizon was in the range 1.9–3.5 mg kg^{-1}. Its highest value is increased at 11.6 fold to the reference value. The content of Pb in potential available form was in the range 100.4–253.6 mg kg^{-1} in B horizon. Risky elements have ability to get from the foodstuffs from soil, water and air as contaminants, some of them could be natural compounds of foodstuffs. By evaluation of grinding fractions from the standpoint of heavy metals we have found out that the limit value of Co has been exceeding. The value of Co was in the range 0.047–0.287 mg kg^{-1} the area of Humenné. The highest acceptable amounts exceeded in the I. and IV. milling fractions. The limit value has been enhanced at 218.5 %. The content of Pb was 2.5 fold higher than the limit value in the analyzed wheat grain. The cultivated winter wheat is not suitable for food processing from the point of Foodstuff Codex.

Conclusions

The contamination in the monitored area has an anthropogenic character which is caused by the industrial emissions produced by chemical factories situated in the surroundings Strážske and Humenné. With the observation of pseudototal (total) and potentially mobile contents of the risky and trace elements in area of Humenné, we can state that the contents of risky elements in A horizon exceeded in the case of Cd,

Ni, Co and Zn. The pseudototal contents of the determined elements show the local enhancement of all heavy metals in B horizon. The potentially mobilized forms of the risky elements were assessed in 2M HNO_3 these limit values exceeded in horizons A and B in the case of Cd and Pb. The highest acceptable amounts were increased in the I. and IV. milling fractions.

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P75 DETERMINATION OF CHANGES IN SOIL ORGANIC MATTER CONTENT THROUGH CARBON AND NITROGEN LABILE FRACTIONS

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Introduction

The quantity and quality of soil organic matter (SOM) are the most important characteristics, which influence the sustainable development. Much more sensitive indicators of dynamic changes of C and N are their fractions, labile carbon or potentially mineralizable nitrogen[1]. Techniques for isolating individual carbon fractions are different. Usually there is acid hydrolysis by H_2SO_4 with different concentrations[2] or HCl[3]. Other developed method was a fractionating method of SOM and fractions or substrates of SOM based on the susceptibility to oxidation by permanganate[4]. Modification and standardization of $KMnO_4$ oxidation technique[5] has increased the precision and simplified the technique, using only one concentration of $KMnO_4$, thereby dividing soil carbon into labile (C_L) and non-labile (C_{NL}) carbon. Research focuses on possibilities of SOM changes evaluation through the C_T – total organic carbon, C_L – labile carbon, C_{NL} – non-labile carbon, L_C – lability of soil organic carbon, LI_C – lability index of carbon, CPI – carbon pool index, CMI – carbon management index, N_T – total nitrogen, N_L – potentially mineralizable nitrogen, N_{NL} –non-labile nitrogen, L_N – lability of soil nitrogen, LI_N – lability index of nitrogen, NPI – nitrogen pool index, NMI – nitrogen management index and selection of suitable parameters for sensitive reaction on SOM changes also in agro-ecosystems.

Table I
Mean values of parameters of SOM quality

	Farming system		Plot	
	ESa	ISb	5	7
LC	0.216[ac]	0.229[a]	0.224[a]	0.222[a]
LIC	1.045[a]	1.170[a]	1.100[a]	1.117[a]
CPI	1.076[a]	1.182[a]	1.162[a]	1.095[a]
CMI	1.126[a]	1.380[b]	1.274[a]	1.232[a]
LN	0.109[a]	0.078[a]	0.103[a]	0.085[a]
LIN	2.304[a]	1.167[a]	2.079[a]	1.392[a]
NPI	1.090[a]	0.997[a]	1.132[a]	0.955[a]
NMI	2.542[a]	1.123[a]	2.359[a]	1.306[a]
		[g kg^{-1}]		
CT	12.885[bc]	11.422[a]	12.205[a]	12.102[a]
CL	2.289[a]	2.122[a]	2.220[a]	2.191[a]
CNL	8.963[a]	9.299[a]	8.352[a]	9.910[a]
		[mg kg^{-1}]		
NT	1338.2[a]	1168.3[a]	1341.7[a]	1164.8[a]
NL	129.2[b]	100.2[a]	122.5[a]	106.8[a]
NNL	1209.0[a]	1068.2[a]	1219.2[a]	1058.0[a]

[a]Ecological farming system,
[b]Integrated farming system,
[c]Values followed by the same letter within each column are not significantly different at $P<0.05$

Experimental

The studied territory of Malanta (lat. 18°08'N and long. 18°08'E) is located in the lower part of Selenec creek basin and its tributaries which belong to the central part of Nitra river basin. The geological substratum is created of few existing rocks with high quantities of fine materials. The soil is Orthic Luvisol. The average annual temperature of air was 9.6 °C and mean annual precipitation was 633 mm. The project with ecological (ES) and integrated (IS) farming systems was established in 1990. We collected the soil samples in the period 2005–2007. We determined C_T[6], C_L[4], N_T[7] and N_L[8] in soil samples. We calculated C_{NL}, L_C, LI_C, CPI and CMI[5]. We used this procedure for evaluating changes of soil nitrogen, as well. Data were analyzed using analysis of variance and differences were determined using the Duncan test. We used correlation to determine the relationships between studied parameters.

Results

Statistically significant higher average contents of C_L were determined in ES than IS. Higher content of C_L was in fertilized variants than in variants without fertilization (Table I).

In our study contents of phosphorus ($r = 0.599$, $P<0.01$) and potassium ($r = 0.488$, $P<0.05$) had statistically significant influence on C_L. L_C was higher in fertilized variants (0.223) than in variants without fertilization (0.202). L_C was in negative correlation with pH_{KCl} ($r = –0.452$, $P<0.05$). Values of LI_C were in correlation with pH_{KCl} ($r = –0.471$, $P<0.05$). On average, statistically significant higher average CMI value was in IS (1.38) than in ES (1.13), which showed on higher changes in organic carbon sources in ES. CMI values showed whether dominant processes are carbon losses or processes of new organic substances production. Statistically significant differences of N_L contents were also found between farming systems. On average higher N_L content was in ES 129 mg kg^{-1} than in IS 100 mg kg^{-1}. Values of N_L were in negative correlation with base exchangeable cations ($r = –0.416$, $P<0.05$) and degree of saturation ($r = –0.404$, $P<0.05$). Content of N_{NL} was in positive correlation with content of phosphorus ($r = 0.564$, $P<0.01$) and potassium ($r = 0.664$, $P<0.01$).

Conclusions

The results focused on the necessity of application, predominantly of carbon and nitrogen fractions on the evaluation of quality changes and losses of SOM. According to statistical assesment suitable parameters for sensitive reaction on SOM changes in agro-ecosystems seems to be mainly parameters C_L, L_C, CMI and N_L.

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P76 DISTRIBUTION OF HEAVY METALS IN SOILS

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Introduction

The soil quality is derived from its loading by hazardous substances. The loading of soil occurs when the soil is not able to lower the negative influences of the heavy metals. The reference value, which represents natural content of heavy metals in soil, forms the basis for evaluation of the content of heavy metals in soil. The important parameters for the input of heavy metals from soil into plants are: the soil reaction, the content and the quality of organic matter, the nutrition of plants, cation exchange and sorption capacity, the microbiological activity, the oxidation and reduction potential, the amount and the quality of the clay fraction of soil and the methods of soil cultivation, etc.

Experimental

The hazardous heavy metals were determined in eight soil subtypes. Their choice was concentrated on the lowland regions (Trnovec n/V. – haplic chernozems, Imeľ – eutric fluvisols, Čičarovce – luvic stagnosols, Dolný Štál – mollic fluvisols, Veľké Leváre – haplic arenosols, Malanta – haplic luvisols). We took four parallel soil samples to be able to examine the soil homogenity of the monitored area. The samples from the pedological probe were taken from the depth of 0–0.1 m; 0.20–0.30 m; 0.35–0.45 m. The state of the soil hygiene was examined by evaluation of the total contents of Cd, Pb, Cr, Cu and Zn. The total contents were determined after the mineralization by wet way H_2SO_4, HNO_3 and $HClO_4$ using the method of atomic absorption spectrometry. We also determined the heavy metals in the pedological probe to be able to estimate the anthropogenical and geochemical origin of the heavy metals. The above mentioned heavy metals were determined in the extract of 2M HNO_3 and in cold. The mobilizable forms of heavy metals were determined in the extract with 0.05M EDTA. The mobile heavy metal forms were determined in extract of 0.01M $CaCl_2$.

Results

The valid legislation was used for the evaluation of soil hygiene. The evaluation is influenced by determination of the total contents of heavy metals and by the determination of heavy metals in 2M HNO_3 leach. Contents of heavy metals in 2M HNO_3; 0.05M EDTA and 0.01M $CaCl_2$ and the percentage abundance of Zn, Cu, Cr, Pb and Cd in individual extractants. The following order of extractability for individual extractants is evident. The results of determination of actual mobilizable forms are considered to be the most acceptable. The percentage contents of individual heavy metals and the total content of heavy metals in highly contaminated soil are as follows: Zn 1.7–13.9 %; Cu 14.1–62.6 %; Cr 0.1–1.3 %; Pb 14.6–24.1 %; Cd 12.9–22.8 %. EDTA and natrium and ammonium of EDTA are able to form stable and defined complexes with heavy metal cations and they cause the solubility of carbonates and oxides Fe and Al. The exctracted contents of heavy metals were measurable during the use of flame AAS. The mutual interactions of Zn, Cu, Cr, Pb and Cd with the soil components influence the pH value, content and quality of organic substances. Apart from the above mentioned soil properties, there are many other variable soil properties. The lowest Zn solubility was determined in subtypes of luvic stagnosols and eutric cambisols with pH values in the acid part and the quality of mould expressed by the ratio of humic acids to fluvic acid was the lowest but on the other hand the percentage of mould has one of the highest values. The solubility of Cu in 2M HNO_3 and in 0.05M EDTA was in all soil subtypes the highest and it is especially valid for haplic arenosols, haplic chernozems, mollic fluvisols (FL_m). The order of solubility for the determined elements and and extractants was as follows: 2M HNO_3 > 0.05M EDTA > 0.01M $CaCl_2$.

The lowest amount of Cd and Pb from haplic arenosols (RM_g) were extracted by extraction with 2M HNO_3 and 0.05M EDTA and the highest amounts of Cd were obtained from mollic fluvisols, haplic chernozems. The evaluation of heavy metal contents extracted from individual extractants at different pH values, the content of mould and its qualitative composition is not unequivocal and dependence between variable soil properties and conditions of environment disappear.

The solubility of Cu in 2M HNO_3 and in 0.05M EDTA was in all soil subtypes the highest and it is especially valid for haplic arenosols, haplic chernozems, mollic fluvisols. The order of solubility for the determined elements and and extractants was as follows: 2M HNO_3 > 0.05M EDTA > 0.01M $CaCl_2$. We think that the best extractant is 0.05M EDTA with restriction to Cd, Pb, Cu and partially for Zn. It is not possible to find an universal extractant for evaulation of heavy metals mobility in soil.

Conclusions

*(i)*The extractability for individual ectractans is evident: 2 mol dm^{-3} HNO_3 : Cu > Cd > Pb > Zn > Cr; 0.05 mol dm^{-3} EDTA: Cu > Cd > Pb > Zb > Cr; 0,01 moldm^{-3} $CaCl_2$: Cd > Cu > Pb > Zn > Cr. *(ii)* It is necessary to emphasize the differences in solubility between the highly contaminated eutric cambisols of Stredný Spiš and other analyzed soils. The different solubility is in all extractants and heavy metals except the solubility of chromium in 0.05M EDTA and 0.01M $CaCl_2$. It is probably connected with the high portion of heavy metals of immissional origin. The other analyzed soil subtypes have a low solubility of Zn in HNO_3 and EDTA in luvic stagnosols.

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P77 MULTICOMPONENT MICRODETERMINATION OF ARSENIC, ANTIMONY, TELLURIUM, SELENIUM BESIDES OF THALLIUM BY ICP-MS IN WATERS

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Introduction

The concentration of As and Tl in the environment is controlled by strict guidelines. Since considerable affection of the human organism is described for As and Tl[1,2]. Te is more toxic than Se but little is known about its requirements. Se is longely known for its ambivalency and particular essentiality for the human and animal organism and its implication in various enzymes on trace levels. Arsenic compounds are an important dopant for the semiconductor silicon production and a modifier of mechanical properties in lead and copper alloys. Complicated hydrolytic equilibria can be present in dilute aqueous solutions in dependence on pH[3].In fact, these equilibria in aqueous solutions have little influence on the results of ICP-MS only. The multicomponent microdetermination of inorganic As, Sb, Se, Te and Tl with ICP-MS is remarkable selective and sensitive and has not been studied in detail[4].

Experimental

C h e m i c a l s

Standard solutions of Se, Te, As, Sb and Tl with 100 μg dm^{-3} were prepared by dilution from original solutions containing 1.000 ± 0.002 g dm^{-3} metals which were purchased from Analytica s.r.o., Prague.

A multicomponent standard containing 1.000 ± 0.002 g dm^{-3} Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, Ti, Zn was also from Analytica s.r.o. Prague.

The tuning solutions for ICP-MS were 1 mg dm^{-3} of Ce^{3+}, Li, Y, and Tl^{+} in 2% HNO_3. In such solution must be the ratio $CeO^{+}/Ce^{+} \leq 1.5$ % and $Ce^{2+}/Ce^{+} \leq 3$ % for bivalent ions. The solution containing 1 mg dm^{-3} of Co^{2+} in 1% HCl was used for tuning with the Helium collision cell.

I n s t r u m e n t a t i o n

An ICP-MS spectrometer Agilent 7500ce Japan was used with a plasma generator of 27.12 MHz and the power output 1,500 W. The sample nebulized by a concentric silica nebulizer MicroMist™ with a cooled Scott chamber entered by an injector of 2.5 diameters into the plasma.

The flow of the carrier argon trough the nebulizer was 1 dm^3 min^{-1} and contained the make up argon 0.33 dm^3 min^{-1}. A constant temperature 2 °C of the nebulising chamber was maintained.

Results

No polyatomic interferences were observed for selected isotopes ^{75}As, ^{82}Se, ^{121}Sb, ^{125}Te and ^{205}Tl such as Ar^{2+}, ArH^{+}, ArO^{+} and ArN^{+}. Six-points calibration plots for selected element isotopes were strictly linear for concentrations less than 1,000 μg dm^{-3} in solutions containing 0.5% HNO_3. The signal intensity considerably decreases with the increasing concentration of acids. For the hydrochloric acid the decrease is 6 % for ^{205}Tl and 11% for ^{75}As with 5% HCl, With HNO_3, the decrease is 30% for ^{82}Se and ^{125}Te but for ^{75}As and ^{205}Tl 15%. The medium of 0.5% HNO_3 is optimal and recommended for the measurement.

I n t e r f e r e n c e s

The effect of 1–250 mg dm^{-3}of Na, K, Ca, Mg, Al, Fe(III), on the signal intensity was evaluated for 100 μg dm^{-3} in 0.5 % HNO_3. For 1–10 mg dm^{-3} of the matrix element the error for the microelement signal does not exceed 5 %. For 50 mg dm^{-3} of the matrix element, the error for the microelements increased to 15–20 % in the presence to Ca, Mg, Al and Fe(III). On the other hand, 250 mg dm^{-3} of Na and K cause less than 10% error for 100 μg dm^{-3} As, Se, Sb, Te, Tl.

In the presence of 1–100 μg dm^{-3} of multicomponent solution containing Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, Ti, Zn with 100 μg dm^{-3} of ^{75}As, ^{82}Se, ^{121}Sb, ^{125}Te and ^{205}Tl a considerable interference was observed and the signal decreased up to 70 %. The error can be decreased in the presence of 100 μg dm^{-3} of internal standard. ^{72}Ge was suitable for ^{75}As in the He mode and ^{82}Se in the normal mode and ^{209}Bi was suitable for the remaining elements measured in normal mode (Fig. 1.).

A p p l i c a t i o n o f W a t e r S a m p l e s

Five-point strictly linear calibration plots with spiked microelements were carried out for all kinds of waters. The slopes of the regression lines were compared with that of ultra pure water to evaluate the influence of the matrix which cause some signal decrease by 5 %. This error can be diminished by using internal standards especially for sea and mine waters.

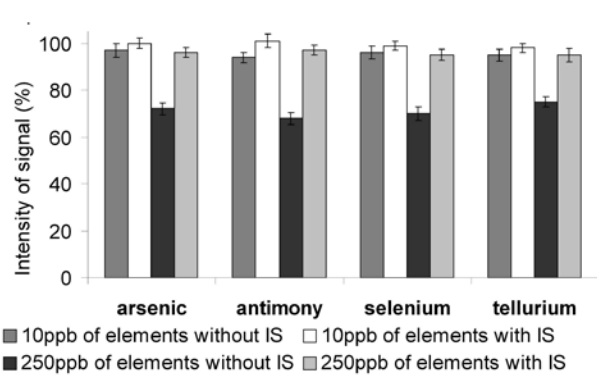

Fig. 1. **The influence of multicomponent standard on the intensity of signal of 100 μg dm^{-3} of ^{75}As, ^{82}Se, ^{121}Sb, ^{125}Te and ^{205}Tl in the presence or absence of internal standard (^{72}Ge and ^{209}Bi)**

The detection limits were expressed according to UPAC[5]. For ^{75}As was the limit of detection 0.05–0.08 μg dm^{-3}, for ^{82}Se 0.04–0.03 μg dm^{-3}, ^{121}Sb 0.03–0.01 μg dm^{-3}, ^{125}Te 0.3–0.02 μg dm^{-3} and for ^{205}Tl 0.005–0.2 μg dm^{-3}.

The evaluation of the contents of microelements resulted form spikes 1, 3 and 5 μg dm^{-3} in solutions containing 0.5% HNO_3. The proper contents of the microelements in the sea and mine waters resulted from the corrected calibration plot by the standard addition method since their contents is higher than the detection limit of these elements.

For the drinking, surface and mineral waters, the evaluation was realized directly from the spikes, comparing the corrected regression equation of the calibration plot. This was because the amounts of microelements were below their detection limit.

Selected internal standards ^{72}Ge for ^{75}As in the He mode and ^{82}Se in the normal mode and ^{209}Bi for the remaining elements, measured in normal mode were always used during evaluation. The confidence intervals according to Dean and Dixon statistics[6] for triplicate analyses were used.

Table I
Evaluation of results from 1, 3 and 5 μg dm^{-3} spikes used

	Drinking water	Surface water	Mineral water
^{205}Tl	1.004 ± 0.005	3.006 ± 0.010	5.004 ± 0.009
^{125}Te	1.003 ± 0.004	3.004 ± 0.009	5.005 ± 0.012
^{121}Sb	1.003 ± 0.004	3.003 ± 0.008	5.003 ± 0.009
^{82}Se	1.003 ± 0.002	3.004 ± 0.006	5.004 ± 0.007
^{75}As	1.001 ± 0.003	3.001 ± 0.005	5.001 ± 0.004

Conclusions

^{75}As, ^{82}Se, ^{121}Sb, ^{125}Te and ^{205}Tl were determined in the concentration range ≤1,000 μg dm^{-3} in solution with 0.5% HNO_3 in the absence and presence of internal standards ^{72}Ge for ^{75}As in the Helium mode, ^{72}Ge for ^{82}Se in the normal mode and ^{209}Bi for ^{121}Sb, ^{125}Te and ^{205}Tl when the normal mode was used. 100 μg dm^{-3} of the microelement can be determined in the presence of 50 mg dm^{-3} of Ca, Mg, Al, Fe(III) with 15–20% error but in the presence of 200 mg dm^{-3} of Na, K with 10% error only. In the presence of 250 mg of a multicomponent sample with 100 μg dm^{-3} of studied elements the signal decrease. The error was decreased in the presence of 100 μg dm^{-3} of internal standard.

Table II
Evaluation of results by the method of standard deviation in the sea and mine water[a]

	Sea water	Mine water
^{205}Tl	0.017 ± 0.025	0.811 ± 0.036
^{125}Te	0.022 ± 0.019	0.421 ± 0.032
^{121}Sb	0.020 ± 0.013	0.229 ± 0.029
^{82}Se	0.054 ± 0.015	1.012 ± 0.014
^{75}As	0.020 ± 0.033	0.537 ± 0.041

[a]Five-points calibration plots used

For water samples the standard addition method was used for sea and mine water only to evaluate the microelements because the concentration of microelements exceeds the practical detection limits from the IUPAC recommendation. For surface and potable waters with the amounts of microelements below the detection limit three spikes were directly evaluated in triplicate according to Dean and Dixon statistics in the presence of internal standards.

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P78 SEPARATION AND PRECONCENTRATION OF ARSENIC, ANTIMONY, SELENIUM AND TELLURIUM ON MODIFIED SILICAGELS FOR THEIR DETERMINATION BY ICP-AES

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Introduction

The determination of toxic or ambivalent microelements arsenic, antimony, selenium and tellurium in water samples requires inevitably a preconcentration prior to the determination by ICP-AES. The separation and preconcentration by various solid phase extractions were earlier studied and such technique widely used for water samples. The complexation of these elements with organic reagents is of particular interest when interacting with various kinds of silica sorbent used.[1–5] The combination of organic reagent with cationic surfactant was examined for sorption in this paper.

Experimental

Chemicals

All chemicals and solvents used were of analytical grade quality.

Astasol standards for arsenic, antimony, selenium and tellurium containing 1.000 ± 0.002 g dm^{-3} of element were from Analytica™ Prague, Czech Republic.

The cationic surfactant 1-ethoxycarbonylpentadecyltrimethylammonium bromide (Septonex®) from Aventa, Czech Republic and organic reagents 4-(2-Pyridylazo)resorcinol (PAR), Pyrrollidincarbodithioate (APDC), thiourea (THU) and 1,2-dihydroxybenzene (PYR) from Lachema, Czech Republic, diethyldithiocarbamidate (DTC) from Fluka, Switzerland and 8-hydroxyquinoline-5-sulphonic acid (8-HQS) from Aldrich, Germany were used.

Modified sorbents were Separon™ SGX C18, C8, SGX NH_2, SGX CN, SGX RPS and SGX Phenyl with particle size 60 μm, from Tessek™ Prague, Czech Republic.

Instrumentation

An echelle based ICP-spectrometer with a prism-predisperser IRIS AP™, (Thermo Jarell Ash) and CID detector with 512 × 512 pixels for 195–900 nm, axial plasma discharge of 1.35 kW and echelle grating with 54.4 lines were used.

The following spectral lines [nm] As 228.8, Sb 231.1, Se 190.6 and Te 214.2 were suitable for final evaluation only because of their high intensity, selectivity and low background influences.

Results

Sorption of Elements on the Silica Sorbent in the Presence of Surfactant

Prior to the sorption, the column was conditioned successively with 10 ml of distilled water and 10 ml of 5×10^{-4}–$\times 10^{-2}$ mol dm^{-3} aqueous solution of surfactant. 50 ml of solution containing 1 mg dm^{-3} of As, Sb, Se and Te (each of them) was always sorbed by a flow rate 1.0–3.0 ml min^{-1} at pH 7. The column was then rinsed with 10 ml of distilled water and the elements eluted with 10 ml of acetone-ethanol (1 : 1) mixture in the presence of 0.1 mol dm^{-3} HCl which showed the highest elution efficiency. The organic eluent was always removed by evaporation under an infra-red lamp to 1 ml in a suitable Teflon dish.

5×10^{-3} mol dm^{-3} Septonex® is optimal for the retention of inorganic form of As, Sb, Se and Te. The recovery values decrease from concentration larger than 1×10^{-2} mol dm^{-3} Septonex® because of the competing influence of micelles formed under these conditions in solutions. The recovery was nearly 100 % on SGX C18 (C8) for Sb, Se and Te but for As it reaches 60 % only. On the other sorbents, the sorption efficiency decreased. As was retained from 4 % on SGX Phenyl to 15 % on SGX CN. 70 % retention of Sb was on SGX NH_2. The recoveries for Se were about 90 % for SGX NH_2, RPS and Phenyl. On the other hand, more than 40 % of Te was retained on sorbent SGX RPS and NH_2.

Effect of Organic Reagents

The retention of monitored microelements was carried out from 50 ml of solutions containing 1 mg dm^{-3} of each metal with organic reagents when the column was previously conditioned by 5×10^{-3} mol dm^{-3} Septonex® only.

The sorption from solution containing 0.85×10^{-4}–3.35×10^{-4} mol dm^{-3} PAR was quantitative for Separon™ SGX C18, SGX C8, SGX CN for Sb, Se and Te. On SGX C18 and C8 the retention of complexes was nearly 100 % for 2.77×10^{-4}–1.1×10^{-3} mol dm^{-3} 8-HQS and also 6.25×10^{-4}–8.33×10^{-3} mol dm^{-3} PYR. The recoveries about 90 % were observed for 4.86×10^{-4}–7.29×10^{-4} mol dm^{-3} APDC and about 80 % for 4.90×10^{-4}–1.50×10^{-3} mol dm^{-3} DTC or 1.05×10^{-3}–4.20×10^{-3} mol dm^{-3} THU for Sb, Se and Te. The sorption of As was far from being quantitative. 1.70×10^{-4} mol dm^{-3} PAR can be used for the retention of As, Sb, Te on SGX NH_2 and SGX RPS for As, Sb, Se. On the other hand, 2.77×10^{-4} mol dm^{-3} 8-HQS is optimal for Se on SGX NH_2 and SGX Phenyl and 2.43×10^{-4} mol dm^{-3} DTC was suitable for the retention of Te on SGX RPS, SGX Phenyl and for As on SGX Phenyl.

Effect of Sample Volume

The influence of sample volume was tested for the retention from 50–1,000 ml solution containing 0.2–0.01 mg dm^{-3} each of element in the presence of the particular organic reagents after conditioning. This corresponds to a 5–100-fold

enrichment of As, Sb, Se and Te which enables the final use of ICP-AES.

Volumes of up to 1,000 ml have no effect on the retention efficiency on Separon™ SGX C18, C8 and SGX RPS. The sorption is however quantitative from 500 ml only on SGX NH_2, SGX CN and SGX Phenyl. The weakening of the retention forces of ionic associate or complexes on the surface of sorbent may supports the subsequent washing out of the element species from the column.

Application for Water Samples on Separon™ SGX C18

Standards of folowing elements were spiked to equilibrated drinking, mineral and river water samples containing no detectable amounts of these elements. The Separon™ SGX C18 was previously conditioned by 10 ml of distilled water and 10 ml of 5×10^{-3} mol dm^{-3} Septonex®. The sorption was provided from 250 ml of sample solutions in the presence of 1.68×10^{-4} mol dm^{-3} PAR.

Table I
The recovery (%) of arsenic and antimony in water samples[a]

Spikes [mg dm^{-3}]	$c_{element}$[b] [mg dm^{-3}]	Arsenic	Antimony
Mineral water			
0.25	0.01	58.30 ± 2.44	98.74 ± 2.70
0.50	0.02	60.02 ± 2.60	101.3 ± 3.40
1.00	0.04	60.23 ± 3.36	99.54 ± 2.87
River water			
0.25	0.01	59.71 ± 3.14	100.9 ± 2.39
0.50	0.02	60.90 ± 2.69	97.26 ± 3.53
1.00	0.04	62.34 ± 2.43	100.9 ± 3.09

[a]The analysis was carried out in triplicate and evaluated according Dean and Dixon[6]
[b]Concentration in 250 ml of water sample

Table II
The recovery (%) of selenium and tellurium in water samples[a]

Spikes [mg dm^{-3}]	$c_{element}$[b] [mg dm^{-3}]	Selenium	Tellurium
Mineral water			
0.25	0.01	100.5 ± 2.57	100.5 ± 3.06
0.50	0.02	98.60 ± 3.00	99.50 ± 3.07
1.00	0.04	99.23 ± 2.66	99.78 ± 2.33
River water			
0.25	0.01	99.78 ± 2.71	100.8 ± 2.35
0.50	0.02	98.90 ± 2.80	99.64 ± 2.47
1.00	0.04	101.7 ± 2.11	100.2 ± 1.73

[a]The analysis was carried out in triplicate and evaluated according Dean and Dixon[6]
[b]Concentration in 250 ml of water sample

Conclusions

The separation and preconcentration of arsenic, antimony, selenium and tellurium in the presence of 1.68×10^{-4} mol dm^{-3} 4-(2-Pyridylazo) resorcinol after previous conditioning with 5×10^{-3} mol dm^{-3} Septonex® was described in this paper. This procedure was successfully used for determination of these elements by ICP-AES in real water samples.

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P79 TREATING WASTEWATER BY USING OF BIOCERAMIC FILTERS

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Introduction

Nowadays, the control and treatment of industrial effluents has become one of the most important steps of the productive process, since the regulatory offices have been very rigorous about this subject[1].

Heavy metals are a group of contaminants that are high toxic to humans, animals, and aquatic lives and are commonly found in many munic pal and industrial wastes[4]. The toxicity of copper released into the environment has triggered a number of studies aimed at its removal from aqueous solutions. As a treatment approach, sorption in columns has widely been used in water treatment[2]. The using of bioceramic filters based on iron oxides, quartz sand, clay minerals, and bacteria could be an alternative way to remove heavy metals from industrial effluents. Iron oxides, a common constituent of soils, sediments, and aquifers, have high surface areas and are capable of adsorbing a significant quantity of metals. They are dominant adsorbents in many environments because of their capability to be finely dispersed and act as coatings on other particles[3]. A number of studies on metal uptake using quartz sands and clay minerals, have been conducted and results have shown good adsorption properties due to its metal-binding capacity and high surface area. Bacteria, in particular, are effiecient sorbents of heavy metals, although subtle differences can be seen between species and under various physicochemical conditions. Previous studies showed that the gram-positive bacteria *Bacillus sp*. was able to retain several heavy metals as silicate minerals or as oxyhydroxides at 20 and 4 °C under laboratory simulations of natural conditions.

The concentration of some of the toxic metals are higher than permissible discharge levels in effluents. It, therefore, becomes necessary to remove these heavy metals from these wastewaters by an appropriate treatment before releasing them into the enviroment.

Experimental

The adsorption of copper by bioceramic filters was studied by column technique. In this study two types of filters were used and compared in sorption efficiency.

Magnetite Preparation

Iron oxides can be easily synthetize in laboratory conditions. Synthetic magnetite (Fe_3O_4) used in this work was prepared by partial oxidation of Fe^{2+} solution at temperature 90 °C under anoxic conditions in the presence of nitrogen ions -oxidizing agent. The surface area was 13 $m^2 g^{-1}$ and particle size range from 0.05–0.2 μm. The main mineral phase was confirmed by RTG diffraction, IR spectroscopy and Mössbauer spectroscopy method.

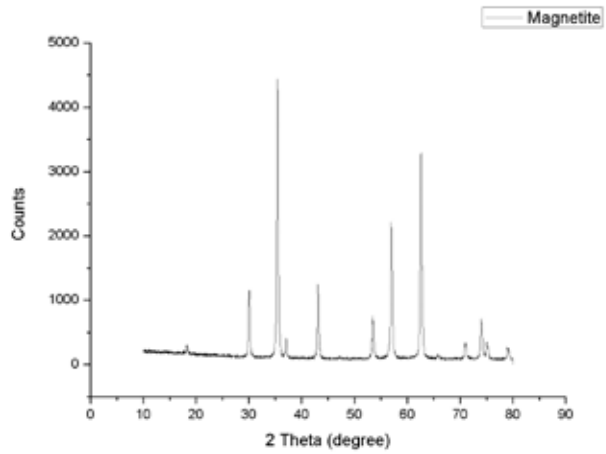

Fig. 1. **XRD-pattern of synthetic magnetite**

Characterization of Quartz Sand and Clay Minerals

Quartz sand used in this work was obtained from Šaštín Stráže deposit (Slovakia) and composed of quartz (88–90 %), feldspar (8–10 %), heavy minerals (1 %) and clay minerals (1 %) of grain size 0–1 mm. The clay mineral used in this study was bentonit composed of montmorillonite (60–80 %) obtained from Jelšový potok deposit.

Table I
Chemical composition of quartz sand

Element [%]	QS
SiO_2	92.7
Al_2O_3	3.95
Fe_2O_3	0.32
TiO_2	0.06
CaO	0.16
MgO	0.15
Na_2O	0.93
K_2O	1.32
Cr_2O_3	0.004
MnO	0.02

Bacteria

In this study two bacterial strains were isolated from the copper pollluted waste water of industry plant: *Bacillus megaterium* and *Pseudomonas diminuta.* The resistance of bacterial strains to copper was tested. The bacterial isolate *Bacillus megaterium* could grow at a concentration ranging from 52–260 mg Cu dm^{-3} and *Pseudomonas diminuta* at a concentration max 52 mg Cu dm^{-3}, at temperature 25 °C. Therefore *Bacillus sp*. isolate was used for this study. The bacterial isolate *Bacillus sp.* was inoculated into flask containing Nutrient broth (Merck) and aerobically cultivated

at 25 °C by the agitating at the speed of 150 rpm. The cells were harvested from the growth medium by membrane filtration (pore size 0.85 μm). Bacteria suspension was prepared with concentration of bacteria 1.2×10^9 ml^{-1} in accordance to MacFarland standards.

Preparation of Copper Model Solutions

The model solution of Cu (II) was prepared by dissolving of $CuSO_4 \cdot 5H_2O$ in deionized water in various concentrations ranging from 0.01; 0.02; 0.04; 0.06; 0.1; 0.5; 1; 1.5; 2; 2.5; 3 to 5 mM. Previous studies showed that at pH 5 is the most effective copper sorption. The initial pH of the solutions was adjusted to 5 by adding 0.1M HNO_3 or 0.1M NaOH for the biosorption experiments. Various Cu(II) concentrations and concentration after sorption process were measured by atomic absorption spectrofotometer (Varian AA240 Z, AA240 FS, Australia).

Column Studies

In this study glass columns were used for experiments. The filtration column was 130 mm high with an inner diameter of 40 mm. Column was packed with appropriate amounts of each sorbent in layers (100 g of quartz sand, 0.5 g of bentonite and 0.1 g of synthetic magnetite). 50 ml of bacteria suspension was passed through the column to adjusted the adhesion of bacteria cells. Two types of filtration columns were prepared. In both types there was 45 mm depth of ceramic medium, one reached with bacteria (biotic filter) and another filter without bacteria medium (abiotic filter). Then 50 ml of Cu(II) model solution was passed through the column at a constant flow rate of 1.5 ml min^{-1}.

Fig. 3. **Sorption columns**

Results

Bioceramic filters composed of quartz sand + bentonite + synthetic magnetite + bacteria were used for the sorption of copper. These two types of filters were compared in sorption efficiency. The effect of initial concentration on the percentage removal of copper by biotic and abiotic filters is shown in Fig. 2. Fig. 2. demonstrates that sorption was more effective in case of biotic filters. The work was carried out at the pH (5) of model solution because this pH value is for copper sorption optimum. The maximum removal of Cu(II) was attained at a concentration 63.5 mg dm^{-3} Cu. High removal efficiency (> 95 %) was obtained over the copper (II) concentration range 1–100 mg dm^{-3}. However the removal percentage decreased with increasing the copper (II) concentration (> 100 mg dm^{-3}). Therefore this method seems to be suitable for the removal of relatively lower concentration of copper.

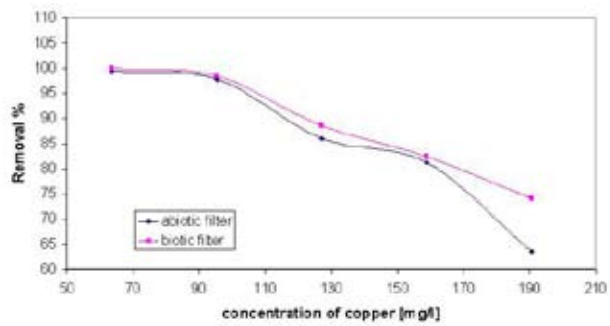

Fig. 2. **Effect of initial copper concentration on percent removal of copper by biotic and abiotic filter**

Conclusions

The present study showed that the bioceramic filters based on quartz sand, bentonite, synthetic magnetite and bacteria can be used as an effective adsorbent of copper. The results of experiments showed high removal of copper by both types of model filters at concentrations ranging from 0.01 to 1.5 mM Cu(II). At higher concentrations removal of copper decreased. The percentage removal was more effective in biotic filters with baceria medium; however the difference compared with abiotic filter was not so marked. Therefore in further experiments bacteria medium with higher concentration of bacteria cells is needed. In the further study various iron oxides (hematite, goethit) and clay minerals (zeolite, kaolinite) will be used in experiments and their sorption efficiency will be compare.

This work has been supported by the Slovak Academy of Sciences (VEGA 2/0049/08).

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P81 DETERMINATION OF SURFACTANTS INCLUDED IN SEWAGE WATER

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Introduction

Surfactants or detergents belong to a group of organic substances that adsorb at a low concentration in the interface, thereby decreasing interfacial or surface energy. Detergents therefore show surface activity which manifests itself by the formation of foam in aqueous solutions[1]. Such properties facilitate the wetting of surfaces and the removal of impurities[2].

Detergents can be divided according to their dissociation properties into the following groups: anionic detergents, cationic detergents, ampholytic detergents, and non-ionogenic detergents[2]. There are many kinds of individual detergents.

During wastewater treatment, detergents account for a high percentage of chemical oxygen demand. Detergents are also able to increase the solubility of other toxic organic components in water and soil. When adsorbed in sludge, they may impair sludge dewatering. Generally, the concentration of detergents at the outflow from a wastewater treatment plant depends on the efficiency and technological parameters of the facility.

Wastewater usually contains different kinds of detergents. For biological treatment, the level of detergents in wastewater should not exceed 1,000 mg dm^{-3}(ref.[4]). In countries where the consumption of washing powders and cleaning agents is high, the concentration of anion-active detergents in municipal wastewater varies in a range of 10–20 mg dm^{-3}. Increased concentrations of detergents can be detected in wastewater from textile industry, the production of washing and cleaning agents and cosmetic production plants, and may exceed 100 mg dm^{-3}. High levels of detergents are also present in wastewater originating from laundries and car washes[5]. The limit concentration of anion-active detergents in drinking water is 0.2 mg dm^{-3}. This parameter is used to indicate the level of pollution in underground water or treated surface water with sewage[2].

Experimental

The samples of wastewater to be analysed were collected at both the inflow and outflow of the wastewater treatment plant of the University of Veterinary and Pharmaceutical Sciences (VFU Brno). The samples of wastewater taken at the outflow were collected before chlorination to prevent the distortion of the results. The sample of water was measured immediately after collection; the transfer of the sample took approximately 10 minutes.

Detergents were determined using Merck spectrophotometric cuvette tests. The method for the determination of anion-active detergents can be used for concentrations ranging from 0.05 to 2.0 mg dm^{-3}. This method is similar to EPA 425.1, US Standard Methods 5540 and EN 903. Cation-active detergents are determined using a spectrophotometric method in a concentration range of 0.05–1.5 mg dm^{-3}. The determination of non-ionogenic detergents was carried out in a range of 0.1–7.5 mg dm^{-3}.

Results

The levels of anion-active, cation-active and non-ionogenic detergents were measured at both the inflow and outflow of the wastewater treatment plant during one week. It follows from the overview of the results provided in Table I that the samples of wastewater contain mainly anion detergents whose level is three orders higher than that of other detergents. The results in the table also demonstrate that the level of detergents in water – particularly anion-active detergents – decreased significantly as a result of wastewater treatment; the highest level detected was 0.97 mg dm^{-3} and was determined on Friday while the lowest level (0.29 mg dm^{-3}) was found on Tuesday. The highest concentration of cationic detergents (0.24 mg dm^{-3}) was detected on Monday and decreased markedly on other days (0.025 mg dm^{-3}). The presence of highly toxic cationic detergents is alarming.

Table I
Comparison of the levels of anion-active detergents in the inflow and outflow [mg dm^{-3}]

	Inflow	Outflow
1	20.00	0.40
2	12.90	0.29
3	18.30	0.49
4	13.90	0.49
5	14.00	0.97

Table II
Comparison of the levels of cation-active detergents in the inflow and outflow [mg dm^{-3}]

	Inflow	Outflow
1	0.400	0.240
2	0.020	0.025
3	0.690	0.025
4	0.025	0.025
5	0.025	0.025

The levels of non-ionogenic detergents in wastewater collected at the outflow were the same in all cases – 0.05 mg dm^{-3}. The differences between individual findings (the highest and the lowest levels) are difficult to explain; it is

possible that other biologically active compounds such as pharmaceuticals may also be present in wastewater affecting the biological stage of wastewater treatment in the respective wastewater treatment plant.

Table III
Comparison of the levels of non-ionogenic detergents in the inflow and outflow [mg dm^{-3}]

	Inflow	Outflow
1	0.69	0.05
2	0.89	0.05
3	2.40	0.05
4	1.40	0.05
5	2.61	0.05

Conclusions

Detergents are closely related to the environment. From an environmental point of view, the impact of detergents on water resources management, their biodegradability, toxicity and eutrophication caused by detergents is very significant. Since a large number of synthetic detergents exhibits insufficient biodegradability, water courses are becoming polluted with these substances. Even low concentrations of surfactants were shown to endanger the organisms in the environment. In order to minimize their concentrations in the environment, generally valid rules should be implemented to help protect our environment.

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P82 EXAMINATION OF THE MUTUAL INTERACTION OF HEAVY METALS IN COURSE OF ADSORPTION FROM MODEL SOLUTIONS

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Introduction

Heavy metals contamination occures in aqeous waste streams of many industries, such as metal plating facilities, mining operations, tanneries etc. Heavy metals are not biodegradable and tend to accumulate in living organisms, causing various diseases and disorders and environmental problems. Treatment processes for metals contaminated waste streams include chemical precipitation, ion exchange, membrane separations (ultrafiltration, reverse osmosis, electrodialysis) and adsorption. Natural materials that are available in large quantities, or certain waste products from industrial or agricultural operations, may have potential as inexpensive sorbents. Due to their low cost, after these materials have been expended, they can be disposed of without expensive regeneration. Cost is an important parameter for comparing the sorbent materials. Adsorption is considered to be the simplest and most cost-effective technique. The removal of heavy metal ions from industrial wastewaters using different adsorbents is currently of great interest[1].

Zeolites are naturally occuring hydrated aluminosilicate minerals. They belong to the class of minerals known as "tectosilicates". The structures of zeolies consist of three-dimensional frameworks os SiO_4 and AlO_4 tetrahedron. This structure causes zeolite to have negatively charged surface. The negative charge is balanced by the exchangable cation (calcium, sodium or potassium). The fact that zeolite exchangeable ions are relatively harmless makes them particularly suitable for removing undesirable heavy metal ions from industrial effluent waters.[2–4]

The zeolite samples from different regions show different behaviour in ion-exchange processes[5]. The ion exchange process in zeolites is influenced by several factors such as concentration and nature of cations and anions, pH value and crystal structure of the zeolite. In this study, the adsorption properties of the natural zeolite and synthetic zeolite Slovakite® with respect to some heavy metal cations in solution were investigated[6].

Experimental

Materials and Chemicals

A natural zeolite was obtained from Slovakia (Nizny Hrabovec). The main phase is clinoptilolite and the chemical compositions are SiO_2 (73.42 %), Al_2O_3 (12.43 %), CaO (2.94 %), K_2O (2.61 %) and Fe_2O_3 (1.05 %). The synthetic zeolite Slovakite® is patented product and the chemical composition is unrevealed by the producent.

Inorganic chemicals were supplied as analytical reagents and deionized water was used. The studied metal ions were Pb^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. Solution of lead and nickel was prepared by using their nitrate salts, $Pb(NO_3)_2$, $Ni(NO_3)_2.6H_2O$. The solution of copper and zinc was prepared from their sulphate salts, $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$.

Adsorption Tests

The ion exchange of heavy metals on natural zeolite and on synthetic zeolite Slovakite® were carried out using the batch metod. Batch adsorption experiments were conducted using 2 g of adsorbent with 200 ml of solutions in flasks containing heavy metal ions of desired concentrations at constant temperature (25 °C). The initial concentration of heavy metals in stock solutions was in the range at 5–1,000 mg dm^{-3}. Sorption experiments were carried out at pH 5.5. The flasks were then agitated in an orbital shaker at a speed of 200 rpm for a period of 2 h. The quantity of elements in solution has been determined both before the introduction of sorbent and after the equilibrium time of 24 hours by AAS.

The amount of adsorbed metal was calculated using the equation:

$$q_{eq} = \frac{c_0 - c_{eq}}{c_s} \quad (1)$$

where c_0 and c_{eq} [mg dm^{-3}] are the concentrations of the metal ion in initial and final solutions and c_s [g dm^{-3}] is the sorbent concentration.

Results and Discussion

Adsorption of Metals on Natural Zeolite

The adsorption of Pb^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} onto natural zeolite as a function of their concentrations was studied at 25 °C by varying the metal concentration from 5 to 1,000 mg dm^{-3} while keeping all other parameters constant. The experimental data were modeled with Langmuir, Freundlich and Redlich-Peterson isotherms. The adsorption isotherms which are the most suitable to fitting the adsorption processes on natural zeolite in single system are shown in Figs. 1. and 2. The isotherm analyses showed different adsorption behaviour for Pb^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. Metal adsorption increased in the following order: $Pb^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+}$ (Figs. 1. and 2.). Fig. 1. illustrates the dynamic adsorption process of Cu^{2+} and Zn^{2+} on natural zeolite. As shown Fig. 1., the maximum sorption capacity of natural zeolite was already exhausted (the equilibrium capacity was achieved) when the metal concentration in solution was in low range. Fig. 2. presents that the sorption capacity of sorbent was not expended even by the highest initial concentration of Pb^{2+} and Ni^{2+} in solution.

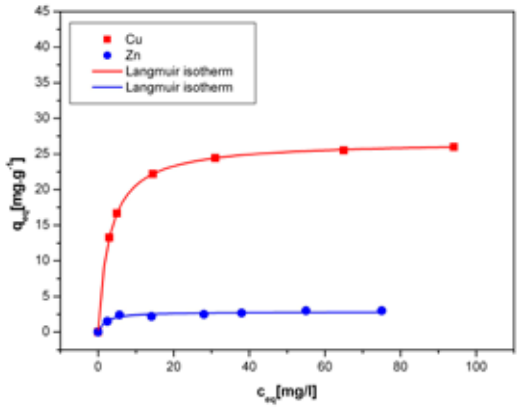

Fig. 1. **Adsorption isotherms of Cu^{2+} and Zn^{2+} on natural zeolite**

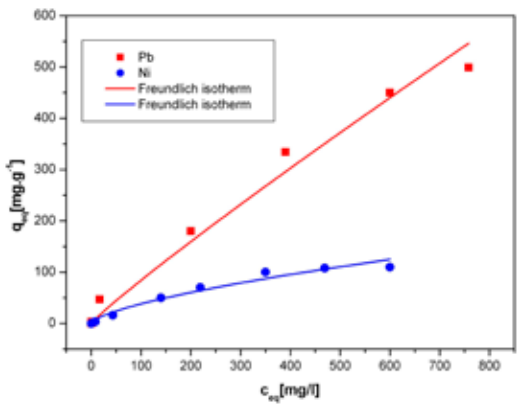

Fig. 2. **Adsorption isotherms of Pb^{2+} and Ni^{2+} on natural zeolite**

The Langmuir and Freundlich models effectively described the sorption data with all R^2 values > 0.95.

Adsorption of Metals on Synthetic Zeolite Slovakite®

The adsorption of Pb^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} onto synthetic zeolite Slovakite® as a function of their concentrations was studied in the same conditions as on natural zeolite. Figs. 3. and 4. illustrate the adsorption isotherms of selected heavy metals on Slovakite® in single component system. Comparing the two isotherms on Fig. 3., Cu^{2+} adsorption is usually higher than Zn^{2+}. For Zn^{2+}, equilibrium adsorption approaches the value of 42 mg g^{-1} while for Cu^{2+} equilibrium adsorption still shows higher value than 130 mg g^{-1}. Fig. 4. shows a comparison of Pb^{2+} and Ni^{2+} adsorption. For both metals, equilibrium adsorption still shows an increasing trend at higher equilibrium concentrations. The cations sorbed from the solutions followed the same order as on natural zeolite but the sorption capacity of Slovakite® was much higher.

The Langmuir and Freundlich models effectively described the sorption data with all R^2 values > 0.98.

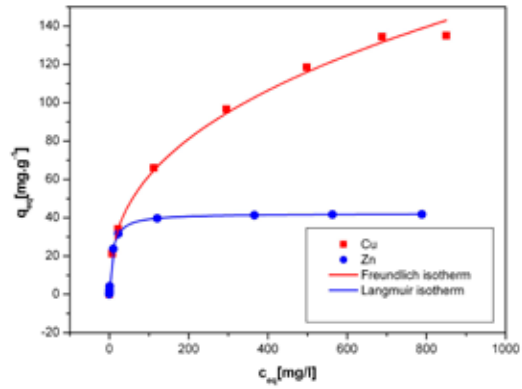

Fig. 3. **Adsorption isotherms of Cu^{2+} and Zn^{2+} on synthetic zeolite Slovakite®**

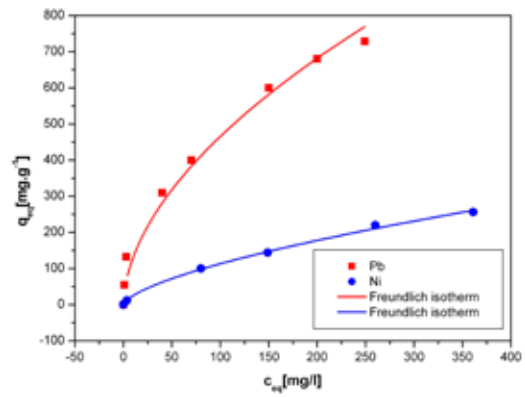

Fig. 4. **Adsorption isotherms of Pb^{2+} and Ni^{2+} on synthetic zeolite Slovakite®**

Conclusions

These results show that natural zeolite from Nizny Hrabovec and Slovakite® can be used effectively for the removal of heavy metal cations from solutions. Best sorption capacity was obtained on synthetic zeolite Slovakite®, decrease in this order $Pb^{2+} > Ni^{2+} > Cu^{2+} > Zn^{2+}$.The selectivity sequence of cations is the same on both sorbents, but the sorption capacity of synthetic zeolite is much higher. The main disadvantage of synthetic zeolite Slovakite® its higher cost.

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P83 FUNGICIDAL EFFECT OF PRINTED TITANIUM DIOXIDE LAYERS

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Introduction

Microorganisms are crucial and inevitable part of life on Earth. They are found basially everywhere – in air, soil, in animal and human bodies, even in places with extreme conditions. Microbial contamination is a serious issue which has to be dealt with in numerous cases of everyday life. Various sterilization and dissinfecting methods have therefore been developed so far.

Photocatalytic processes on thin layers of titanium dioxide represent a new approach to the everlasting struggle against microbial contamination. Reactive oxygen species generated on the surface of irradiated TiO_2 inactivate most type of microbes[1]. Apparently, titanium dioxide coated surfaces self-reducing the population of microbes to minimal level and preventing their growth would be of a great importance.

Most photodegradation reactions on organic substrates are based on the oxidative power of photoinduced electronic holes or are mediated by HO•radicals. Such reaction usually lead to a complete mineralization of organic substrate to carbon dioxide and water. However, it is necessary to provide a reducible reactant (i.e. electron acceptors) which would react with photogenerated electrones. In most cases of photocatalytic degradation reactions, oxygen is present and it acts as primary electron acceptor. Oxygen is thus transformed to superoxide radical ($O_2^{\bullet -}$) and in this way a hydroxyl radical can be produced:

$$TiO_2 \xrightarrow{h\nu} TiO_2(e^-_{cb}, h^+_{vb}) \rightarrow \text{recombination} \quad (1)$$

$$TiO_2(h^+_{vb}) + H_2O_{ads} \rightarrow TiO_2 + HO^{\bullet}_{ads} + H^+ \quad (2)$$

$$TiO_2(e^-_{cb}) + O_{2_{ads}} + H^+ \rightarrow TiO_2 + HO^{\bullet}_2 \leftrightarrow O^{\bullet -}_2 + H^+ \quad (3)$$

Experimental

Material and Methods

Sol and substrate preparation. Sol-gel technique was applied to titanium dioxide thin films preparation using titanium(IV) propoxide as titanium precursors. A mixture of absolute ethanol and acetylacetone (ACAC) was added to titanium(IV) propoxide (TTP) under continuous stirring. Then a small amount of water in ethanol was dropped at last to the previously mixed solution. Soda lime glass plates with sizes of 50 × 50 × 1.5 mm were chosen as a substrate for immobilization of TiO_2 thin films. Soda lime glasses were treated in boiling 9M sulphuric acid. Before the preparation of the thin films, each glass was pre-treated in order to eliminate the dust, grease and other residues using liquid surfactants and dried under air flow.

Sol application was performed in a novel innovative way utilizing a modified office inkjet printer. Ink cartridges were removed from the printer and the ink tubing and printhead were flushed and purged with anhydrous propanol. "Virgin empty" spongeless carts were supplied by MIS Associates, USA. Sol was filtered through 0.2 μm mesh size syringe filter and loaded into one "virgin empty" cart. This cart was installed into the printer in the black position and after a series of head cleaning cycles a perfect nozzle check pattern was obtained. Cleaned glass plates were then mounted into a modified CD holder, fed into the printer and printed with "black only" driver setting. The colour of the printed pattern was varied in different shades of grey (100 %, 95 %, 90 %, 80 %, 70 %, 60 %) and thus glasses with varying sol loading were printed. The resolution, print speed and media settings were also varied and their influence on the resulting TiO_2 layer properties was evaluated. Two way of printer setting were chosen for thin layer of TiO_2 preparation – slow (S) and rapid (R). The sample marked as 100 R corresponds to 100 % of sol loadings printed by rapid way.

Layer treatment. After this procedure, the coated glass plates were dried in the oven at 110 °C for 30 min. Finally, the deposited layers were thermally treated in a calcination furnace at 450 °C for 4 hours.

Photocatalytic Inactivation of Yeasts

A 24-hour culture of yeast *Candida vini* CCY 29-39-3 (provided by Slovak Yeast Collection, Bratislava) was prepared at 25 °C. After the cultivation, 10 ml of culture medium was sampled into a plastic test tube, rinsed twice and centrifuged at 4,000 rpm for 6 minutes. The supernatant was discarted and the yeast sediment was diluted with 1 ml of distilled water an throroughly mixed.

A titanum dioxide coated glass plate was irradiated by UV lamp for 30 minutes in order to obtain a superhydrophilic surface. 25 μl of diluted yeast suspension was pipetted onto the glass plate and evenly spread across its surface. Then the glass plate with yeast suspension was placed in a sreaction chamber. The chamber consisted of a Petri dish with reflective aluminum foil bottom and quartz glass cover. A few drops of water were aplse placed into the reaction chamber in order to maintain the humidity.

The reaction chamber was irradiated by 4 fluorescent lamps Sylvania Lynx-S 11 W with emission maximum at 350 nm. The irradiation intensity was 1 mW cm^{-2} within 290–390 nm spectral region. Irradiated samples were dyed and observed by fluorescent microscopy.

Survival Ratio Calculation

The exposed yeast suspension was mixed with 25 μl acridine orange solution (1×10^{-4} mol dm^{-3}) in phosphate buffer of pH = 6. After thorough mixing, the sample was observed with epi-fluorescent microscope Nikon Eclipse E200.

20 digital images of randomly chosen different places ocross the glass plate were recorded using CCD camera PixeLINK PL-A662 mounted on the Nikon microscope. At every recorded image, the number of living cells N_L (green flurescence) and dead cells N_D (orange fluorescence) was counted. Then, the survival ratio *SR* was calculated:

$$SR = \frac{N_L}{N_{L+D}} \quad (4)$$

Results

After irradiating *Candida vini* suspension deposited on the 100 R glass plate a significant inactivation was observed – the SR dropped to 0.032 ± 0.023 within 70 minutes. On the other hand, the non-irradiated sample showed no inactivation within 70 minutes. These observations are in a good compliance with the results of Seven et al.[4], who also observed no inactivation of microbes on titanium dioxide in darkness. Only very small inactivation was observed on an irradiated bare glass without the catalyst layer. (Figs. 1., 2.).

These results are in agreemnet with the observations made by Kühna et al.[3], who irradiated bacteria *Pseudomonas aeruginosa* on a glass plate covered with titanium dioxide. They found out that bacreial cell inactivation takes place. This phenomenon was explained to be caused by the oxidative stress of oxygen radicals inside cells during the exposure by UV-A radiation. Once the stress rises over a certain threshold, the cell dies.

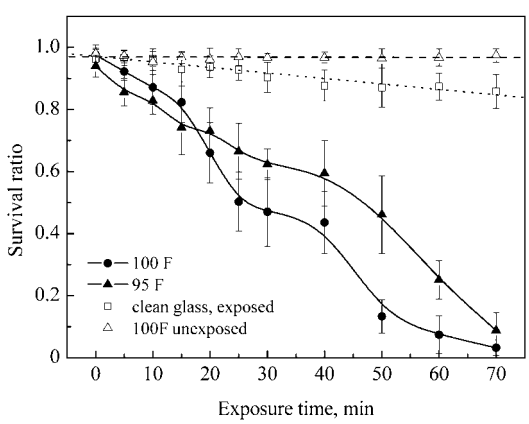

Fig. 1. **SR comparison for *Candida vini* at different conditions on R substrates**

A constant decrease of SR in a certain time from the reaction start was observed by Benabbou et al.[2] in the case of *Escherichia coli*. Cell mebrane damage resulting from photocatalytical processes leads to an increase in membrane permaebility and eventually to free outflow of cell fluids. Therefore both bacterial cells as well as molecules of intracellural organels can become the substrate of reactive oxygen species (ROS) attack. ROS react simultaneously with the cytoplasmatic membrane of living cells and with the remains of dead cells (polysycharides, lipids) at the same time. Our results also confirm this hypothesis.

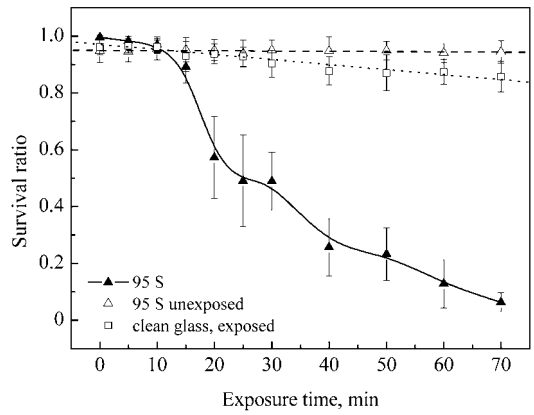

Fig. 2. SR comparison for *Candida vini* at different conditions on S substrate

When we compare the inactivation rates for *Candida vini* on R and S substrates (Fig. 2. and Fig. 1.) it becomes clear that the inactivation rate of *Candida vini* does not depent on the structure and topology of the catalyst layer, as long as the glass surface is well couted by titanium dioxide.

Conclusions

When comparing the photocatylic inactivation rate of *Candida vini* on two types of immobilised catalyst (rapidly printed and slowly printed titanium dioxide layers) it is possible to conclude that significant inactivation was observed on glass plates with very high sol loading, i.e. with very well covered surface (samples 100 R, 95 R, 95 S). We also observe a certain decrease in the inactivation rate after reachicg the SR value of appr. 50 %. This might be caused by the simultaneous consuption of ROS both by the still living cells membrane as well as the organic remains of already killed cells. Almost constant SR value between 25 and 35 minutes suggest a competitive reaction pathway.

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P84 PHOTOCATALYTIC DISINFECTION OF WATER USING Ag/TiO_2

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Introduction

Photocatalytic oxidation of organic compounds represents a major potential to be applied in environmental technologies. The photocatalytic process is capable to decompose most organic matter to water and carbon dioxide. Conventional cleaning technologies such as ozonization or chlorination have their limits. For example, ozone decomposes readily and chlorine, which is used widely for the dissinfection of drinking water, can react with organic compounds to form toxic byproducts[1]. Methods utilizing ozone a UV radiation are expensive and often technically challenging.

Reactive oxygen radicals are are produced in the presence of adsorbed oxygen and electron donor. These radical can cause rapid microbe cell death and at the same time decompose organic compounds.

The fungicidal effect of TiO_2 can be mediated by the presence of a noble metal, such as silver, at the catalyst surface[1]. Therefore, the deposition of silver nanoislets on the catalyst surface can lead to the production of a highly effective dissinfecting agent.

Experimental

Multi-Tube Flow Reactor

Suspension of yeast was colected in 3-neck flask and was circulated with peristaltic pump through tubes irradiated by 4 Sylvania LYNX lamps with total power output 44 W (Fig. 1.). A flow rate was 50 ml min^{-1}. A transmitance of glass wall of tubes was 93 % at maximum light output of Sylvania LYNX lamp at 350 nm. Irradiance was maintaned at 1.0 mW cm^{-2} by lamp distance adjustment.

The inner walls of reactor were covered by titanium dioxide layer using sol-gel process based on tetraisopropoxide solution stabilized with acetoecetone. The inner glass walls were coated by dip-coating method. For experiment with Ag/TiO_2 the metallic silver was deposited on TiO_2 layer by photocatalytic process from silver nitrate solution wih ethanol.

Tubes with 1.2 % mol. and 2.4 % mol. of silver were prepared.

Yeast Suspension and CFU Evaluation

The yeast *Hansenula anomala* CCY 38-1-30, supplied by Slovak Collection of Yeasts, Bratislava, was cultivated in malt extract at 28 °C. After 24 hours a 10 ml was taken into test-tube for centrifugation. The pelets were washed with sterile distilled water and centrifugated. This process was repeated twice. The pelets were resuspended in 10 ml of water and number of cells was calculated using Bürker chamber. This suspension was used for preparation of suspnesions with various cell concentration.

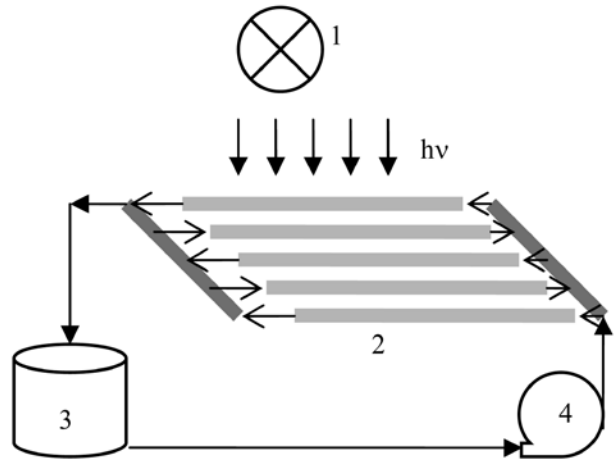

Fig. 1. **Multi-tube flow reactor: 1 – Sylvania LYNX lamps (4 × 11 W), 2 – tubes, 3 – multi-neck flask with yeast suspension (250 ml), 4 – peristaltic pump**

From the multi-tube flow reactor were taken samples in regular intervals. Number of colony forming units (CFU) was evaluated by indirect way: 100 µl of sample was spread on malt extract agar in 3 Petri dishes. After 48 hours of cultivation a number of colonies were enumerated and an average value was expresed as CFU ml^{-1}.

Results

We conducted experiments with photocatalytic inactivation of yeast cells in a multi-tube flow reactor with varying concentrations of cells (500 to 5,000 CFU ml^{-1}). We showed that during the experiment, the number of viable yeast cells decreases. The cell wall of *Hansenula anomala* is quite thick with a rigid structure, therefore we can expect it to be very resistant against ROS attact. Nevertheless, a total cell inactivation was achieved within 160 minutes.

We also observed the photocatalytic inactivation of *Hansenula anomala* (Fig. 2.) on TiO_2 photocatalyst layer overcoated with a very small amount of metallic silver. We also observed a decrease in the size of colonies grown from survivng cells. This phenomenon was noted also by Erkan et al.[6], who described diminishing colonies of *Saccharomyces cerevisiae* after irradiation in TiO_2. In this case, however, the titanium dioxide was overcoated with paladium and the diminishment is believed to be caused by the weakening of surviving cells.

It is well known that pure silver, copper, zinc and their ions pose antimicrobial properties. If these metals are deposited onto TiO_2 thin films, their antimicrobial effect will act together with the antimicrobial effect of bare TiO_2. We can expect the metallic silver to prevent elcetron-hole recombination on the surface of the photocatalyst and thus improve the efficiency of the photocatalytic process. In this case the pho-

tocatalyst would ensure the surface sterility upon the irradiation by UV and metal ions will do their job in the darkness[7]. According to Semikin a Skulachev, the interaciton of Ag^+ a SH groups of respiratory enzymes can lead to cell membrane permeability changes, resulting into major disorders in the whole cell. Apart from the membrane permaebility change, a cell-membrane separation is possible as well. Eventually, the possibility of Ag^+ addition to the bacterial genetic material was reported, too[4].

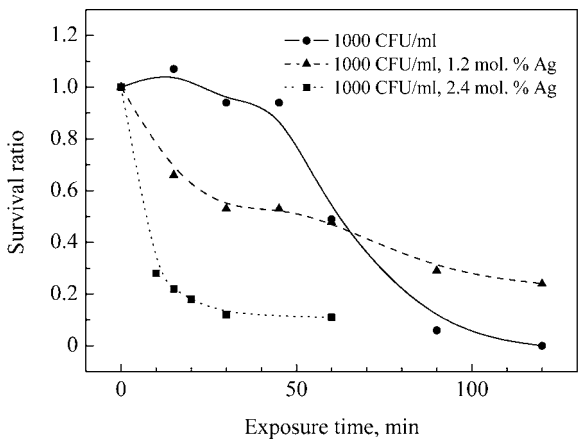

Fig. 2. **Photocatalytic inactivation of *Hansenula anomala* cells on TiO_2 and Ag/TiO_2 layers in multi-tube flow reactor**

We showed that yeast cells of *Hansenula anomala* are not capapble of regeneration, which is otherwise usual, especially in the case of bacteria. The cells are probably damaged in such extend that they loose their reproduction ability. Identical effect was observed on combined surface of Ag/TiO_2 (Fig. 3.).

Conclusions

The collected result indicate that it is possible to photocatalytically inactivate cells even with a very rigid cell wall (*Hansenula anomala*). In this way, a complete photocatalytic dissinfection of water can be performed. A very small amount of metallinc silver deposited on the surface of titanium dioxide photocatalyst enhanced the inactivation rate.

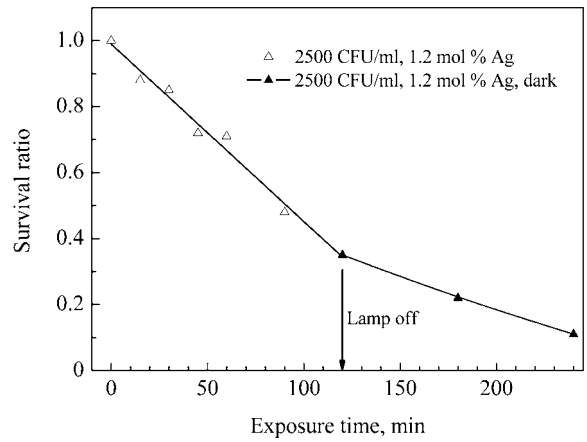

Fig. 3. **Photocatalytic inactivation of *Hansenula anomala* cells on Ag/TiO_2 layer in multi-tube flow reactor. The lamp was powered off after 120 minutes**

We also showed that in the case of *Hansenula anomala*, the surviving cells are not able to reproduce after the irradiation is terminated. The cells lose their reproduction ability. Similarly, the cells lose their reproduction ability after their irradiation on TiO_2 surface doped with silver islets (1.2 a 2.4 % mol. silver).

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P85 LABORATORY STUDY OF ARSENIC MOBILITY IN STREAM SEDIMENTS AND IMPOUNDMENT MATERIAL USING COLUMN EXPERIMENTS

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Introduction

High arsenic contents in the impoundment situated near the village of Poša in the upper part of the catchment of the Kyjov brook in eastern Slovakia, represent an environmental problem because of As mobilization and transport from the impoundment material and significant contamination of surface water of the Kyjov brook (the mean As values of 11,385 $\mu g_{As}\,dm^{-3}$, 1,778 $\mu g_{As}\,dm^{-3}$ and 295 $\mu g_{As}\,dm^{-3}$ measured in the Kyjov brook during 2000, 2005 and 2007 respectively)[1]. Decreasing concentrations of As in surface water are involved in delimited using of impoundment in last few years.

Arsenic distribution in different stream sediment constituents and its mobilization determine As concentration in aquatic environment and affect its bioavailability and toxicity to the biosphere[2,3].

The major processes controlling As leaching from sediments to natural waters include mineral (co)precipitation/dissolution, adsorption/desorption, chemical and biological transformations. The conditions present such as pH, redox potential, solution composition, the sediment properties and mineralogical composition of the sediment determine the dominant processes affecting the environmental fate of As in the stream sediments and ist leaching behaviour[4,5].

The main objective of this study was to investigate leaching behaviour of As from the heterogeneous impoundment material and the three stream sediments and the evaluation of the total As mobility.

Experimental

The samples used in this work are either the stream sediments of the Kyjov brook taken at the distance of 100, 1,000 and 2,000 m from the impoundment (denoted as KY-100, KY-1000 and KY-2000) or the impoundment material (denoted as KY-0).

Continuous column leaching experiments were conducted under standard conditions (25 ± 3°C, 101,325 Pa) to provide information about As release, its binding and desorption kinetic. The experiments were run in duplicates. Each of the two glass columns per one sample was filled with 50 g of a dry sample (a < 1 mm fraction) and columns were during five days flushed with 1.3 dm^3 of 0.0125M solution, that was prepared to resemble the composition of surface waters of the Kyjov brook and contained by 2×10^{-5} M PO_4^{3-}, 2.8×10^{-3} M Cl^- and 3.2×10^{-3} M SO_4^{2-}. The upward flow was regulated at a rate of 0.2 $ml\,min^{-1}$. During the experiment, 14 samples of leachates were collected from each of the columns, pH values were measured and the concentrations of As were determined by graphite furnace atomic absorption spectrometry (Perkin-Elmer 4110 ZL).

Results

The results of the column experiments showed that theAs release from the solid samples was likely controlled by Fe and Mn oxohydroxides, pH values of the geosorbents and leachates and also organic carbon content. It was also observed that the time was an important factor influencing the As release.

Significant correlations of the amounts of As released from the solid samples with its total contents ($r = 0.975$, $P < 0.05$) (Fig. 1.) as well as with total organic carbon contents ($r = 0.942$; $P < 0.05$) were found. The total organic carbon content was measured using a Leco RC-412 multiphase determinator at 550 °C.

The impoundment material (KY-0) has the highest total organic carbon content (37 %), which is likely attributed to the fact that stored sludge consists of fly ashes derived from coal and chemical waste combustion. Alkaline character of the KY-0 (pH = 8.55) as well as high leachate pH (pH ~ 9.0) enhance release of As and its transport to the liquid phase. The results of various studies showed that the amounts of As oxyanions released from different solid geosorbents increase in alkaline conditions, mainly when pH > 8(ref.[6]) The maximum As concentration in column leachates from KY-0 was determinated after 25 hours, when the easily soluble fraction of As weakly bound on the solid surface was released. The late hindered As release from KY-0 could be caused by the presence of mullites and vitreous phases. It is suggested here that some fraction of As may be only hardly available to be released because of its strong binding within the heterogenous components.

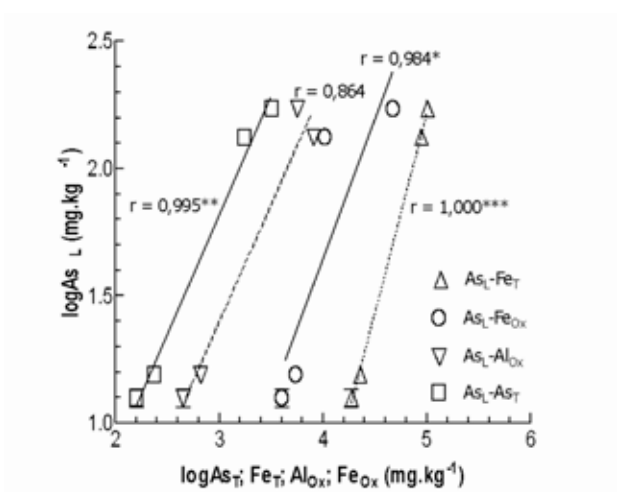

Fig. 1. **Relationships between the amounts of As released and total contents of As, Fe as a ammonium-oxalateextractable Alox, Feox in the log-log form**

Notably high accumulation of trace elements including As in the most contaminated sample KY-100 (3,208 $mg_{As}\,kg^{-1}$) with the lowest relative mobility, is likely due to high contents of the Fe, Al, Mn oxohydroxides. The relative mobility of As was inversely related to the oxalate-extractable Mn content ($r = -0.960$, $P < 0.05$) and not significantly to the oxalate-extractable Fe content such that diffusion in hydrated micropores of amorphous Fe and Mn oxides might be the rate limiting mechanism of the As release (Fig. 2.).

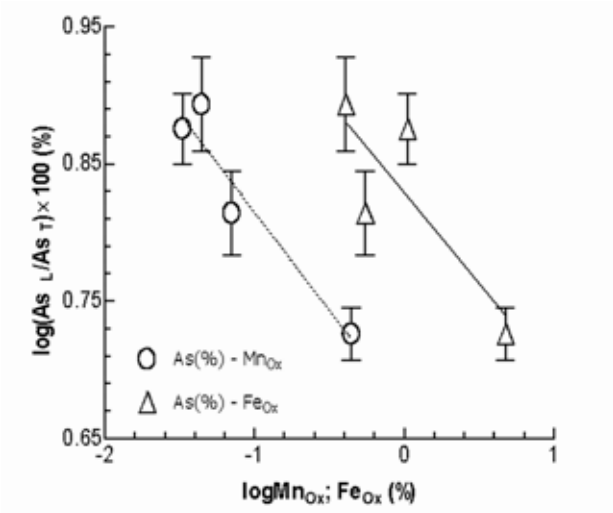

Fig. 2. **Relationship between the relative mobility of As and the ammonium oxalate-extractable Mn, Fe contents in the log-log form**

The amount of As released from samples KY-1000 and KY-2000 increased rapidly within short extraction times, reaching the apparent equilibrium after 174 min. The relative mobility of As in KY-1000 and KY-2000 was approximately the same as in impoundment material although the total Fe, Mn contents in KY-1000, 2000 are 2-times lower as compared with KY-0. The total contents of elements Cd, Cr, Fe, Hg, Mn, Pb, Zn, Al, As and Sb were determined after digestion with acid mixture.

Conclusions

The fractions of As released in column experiments were generally less than 10 % (5.32–7.83 %) of its total contents, but they represented high absolute amounts of readily available and water-soluble As (83 $mg\,kg^{-1}$). The fraction released from the source impoundment material (132 $mg\,kg^{-1}$) represented high As concentration in its leachates, reaching up to 8,000 $\mu g\,dm^{-3}$. As concentrations in the leachates from all the samples exceed maximum permissible level in drinking water (10 $\mu g\,dm^{-3}$; Decision of the Slovak Health Ministry No. 151/2004).

This work has been supported by the Slovak Grant Agency under VEGA project No.1/2037/05.

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P86 ANTIBIOTICS IN THE ENVIRONMENT

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Introduction

Tetracyclines (TC) present a class of antibacterial drugs. Due to their broad antibacterial spectrum and economic advantages, tetracyclines have been commonly used in veterinary medicine and in human medicine for the purpose of prevention and treatment of disease. However, their widespread utilization could lead to TC residues in animal-originated food and in the environment. The antibiotics in food and water consumed for long periods can also cause problems regarding the spread of drug-resistant microorganisms[1].

It is well known that the principal pathway of antibiotics into the aquatic environment is via wastewater systems following consumption and excretion by humans, and via effluents from landfills and farms[2]. Presently conventional wastewater treatment plants (WWTPs) were designed without consideration of antibiotics removal from wastewater. Many previous studies have shown that, while some antibiotics may be eliminated in the WWTPs, some other may be hardly removed in the process, therefore they can reach the aquatic environment[3,4]. Due to irremovable antibiotics, wastewater treatment plants become an important point source of emissions into the environmen[1,2,3].

Experimental

To monitor the tetracycline residues a reliable method is needed. SPE for purification and preconcentration of analytes was used. Spectrophotometric method for antibiotic determination was used.

S a m p l i n g

Waste water samples have been taken from the waste water treatment plant (WWTP) in the University of Veterinary and Pharmaceutical Sciences Brno campus in one-day intervals into amber glass sampling bottles. In this WWTP, we decided to deal with the antibiotics in particular because these drugs samples were taken from the WWTP inlet and compared with the samples taken from WWTP outlet.

The samples were processed immediately or stored in a refrigerator till the following day.

R e a g e n t s

During the process, the following chemicals have been used: oxalic acid, p.a. sulfuric acid p.a., dinatrium phosphate dodecahydrate p.a., all from Lachema, CZ; acetonitrile for HPLC, methanol for HPLC, from Riedel-de-Haen, SRN; citric acid, p.a. Onex, CZ; Chelaton III, p.a. Penta,CZ;

Tetracycline, Chlorotetracycline and Oxytetracycline, standards for HPLC, were from Sigma Aldrich, CZ.

McIlvain buffer: citric acid (12.9 g dm^{-3}), natrium salt of ethylenediaminetetraacetate (37.2 g dm^{-3}), dodecahydrate of dinatrium phosphate (30.2 g dm^{-3}) in deionized water

Elution mixture: 20 mmol dm^{-3} oxalic acid in methanol.

S a m p l e T r e a t m e n t

Samples were filtered using paper filters to remove particles. Volume of 200 ml samples were extracted 20 minutes with McIlvain buffer in an ultrasonic bath.

A Phenomenex Strata C18-E SPE column (55 µm, 500 mg 6 ml^{-1}) was conditioned with 4 ml of methanol followed by 3 ml of McIlvain buffer. After sample loading, the column was washed with 3 ml deionized water. The column was dried using the vacuum of the SPE manifold. Then the tetracyclines were eluted with 10 ml of elution mixture.

S p e c t r o p h o t o m e t r i c A n a l y s i s

The analyses were carried out on UV-VIS spectrophotometer Spectronic Helios (UK). Deuterium and wolfram lamp was used for determination.

At first the absorption spectrum was measured in the range 230–450 nm step by step 2 nm. Tetracyclines have two absorption maximas as resulting from absorption spectrum and with accordance to published data: 246 nm and 360 nm[4]. Spectrophotometer was adjusted to zero value through the use of elution reagent. Subsequently, real samples were measured to determine the absorbance value at absorption maximum. Quartz cuvette with optical path 1 cm was used.

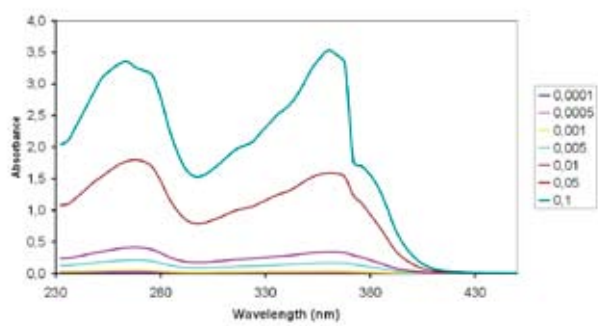

Fig. 1. **Absorption spectrum of tetracycline in various concentrations [mg ml^{-1}]**

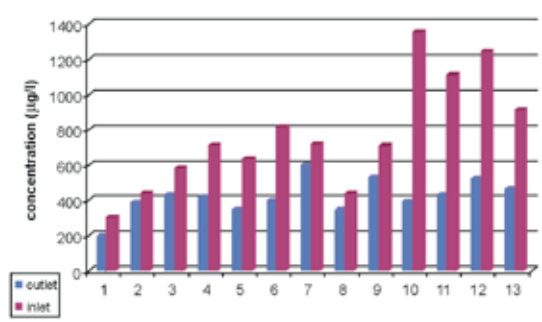

Fig. 2. **Concentration of tetracycline in real samples**

Results

All tetracyclines have typical absorption spectrum. They have two characteristical absorption maximas, 264 nm and 360 nm. Due to their characteristic we could determinate tetracyclines and their metabolites together by spectrophotometry.

Inlet concentrations of analytes were always higher than outlet concentrations. This indicates that these pharmaceuticals are removed partially in the wastewater treatment plant. The change of tetracycline concentration may be caused by photodegradation.

Conclusions

As the use of pharmaceuticals is increasing, fast sample preparation and determination method is required.

Samples were taken from wastewater treatment plant placed in Veterinary and Pharmaceutical university Brno campus, from inlet and outlet.

Samples were filtered before extraction.

SPE was optimized for preparation and preconcentration of wastewater samples.

An efficient method for determination of tetracyclines in wastewater, using spectrophotometry, was developed.

Spectrophotometry was used because this is commonly used in laboratories and machine operation is cheap.

This work has been supported by grant COST, action 636, project no. OC-183.

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P87 THE FLUORIMETRIC DETERMINATION OF ALUMINIUM, GALLIUM AND INDIUM WITH 8-HYDROXYQUINOLINE-5-SULPHONIC ACID IN AQUEOUS AND SUBMICELLAR MEDIUM

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Introduction

The complexing and analytical properties of 8-Hydroxyquinoline-5-sulphonic acid (QSA) are similar to 8-Hydroxyquinoline but the solubility of complexes in aqueous solutions increases and no fluorescence of the reagent in the large pH interval has been observed. The QSA was formerly used as a fluorogenic reagent for a number of metal ions including Mg^{2+}, Zn^{2+}, Cd^{2+}, Ce^{3+} and Al^{3+}(ref.[1]). The fluorimetry of Gallium and Indium complexes in aqueous solutions was only briefly mentioned.

The fluorescence of metal complexes with QSA can be considerably enhanced in the presence of surfactants in submicellar and micellar medium[2,3]. The fluorescence properties of the metal species with QSA were tested in the presence of surfactants and exploited chromatographically in very low concentrations.

Thus the cationic surfactants were widely used for the enhancement of fluorescence of metal complexes with QSA[4]. The attention was also paid to the fluorimetric determination of Al with QSA also in mixtures with Zn using derivative synchronous scan in the presence of surfactant[5]. The Partial Least Square Method (PLS) in combination with pH gradient FIA was used in binary mixtures of ions in complexes with QSA whose fluorescence was enhanced by the surfactant[6].

In this paper the fluorescent Al^{3+}, Ga^{3+} and In^{3+} complexes with QSA were studied in detail in the absence and presence of surfactants and optimal conditions were recommended for the determination of particular metals in the presence of Zephyramine. Normal and first derivative spectra were evaluated.

Experimental

Instruments

Spectrofluorimeter Aminco Bowman, Series 2 with 1 cm quartz cells, 4 nm exit slits, photomultiplier under 450–850 V. Sample cell was tempered to 20 °C.

The calculation of the first derivative was made by using the instrument software using Golay-Savitzky 11-point convolution.

Chemicals

- Standards used, Analytica s.r.o., Prague:

Aluminium chloride 1.000 ± 0.002 g dm^{-3} containing 5 % HCl
Gallium chloride 1.000 ± 0.002 g dm^{-3} containing 10 % HCl
Indium chloride 1.000 ± 0.002 g dm^{-3} containing 10 % HCl

- 8-Hydroxyquinoline-5-sulphonic acid hydrate (QSA), Sigma–Aldrich was used without purification
- 0.1M Benzyldimethyltetradecylammonium chloride (Zephyramine®) – Sigma-Aldrich Co.
- 0.1M 1-ethoxykarbonylpentadecyltrimethylammonium bromide (Septonex®)–Sigma-Aldrich Co.
- 0.1M Dodecylbenzyldimethylammonium bromide (Ajatin®) – Sigma-Aldrich Co.
- 1% Didodecyldimethylammonium bromide – Sigma-Aldrich Co.
- 1% Polyoxyethylene(23) lauryl ether (Brij 35) – Sigma-Aldrich Co.
- 1% Hexadecyltrimethylammonium chloride – Fluka.
- 1% 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (Triton X-100) – Calbiochem Co.
- 0.1 M Sodium dodecylsulphate (SDS) - BDH Chemicals, England.

Evaluation of Data

The limits of detection X_D^{α} and X_D^{β} calculated according to Graham[7] follow from the calibration plots and their confidence intervals were compared with those from multiple measurements of the background according to IUPAC[8].

The stoichiometry of complexes was evaluated by using the modified continuous variations method[9] in the absence and presence of cationic surfactant Zephyramine. The fluorescence was measured for several sums of equimolar solutions, where $c_o = c_M + c_L$.

Results

The complexes of 8-Hydroxyquinoline-5-sulphonic acid with Al, Ga, and In produce an outstanding fluorescence for wavelengths over 430–600 nm with λ_{max} at 495 nm for Al, λ_{max} at 504 nm for Ga and λ_{max} at 519 nm for In. The corresponding excitation λ_{max} are 360 nm, 365 nm and 367 nm. The fluorescence shows considerable pH dependence which reaches the maximum in solution with $c_L = 7.4 \times 10^{-5}$ M

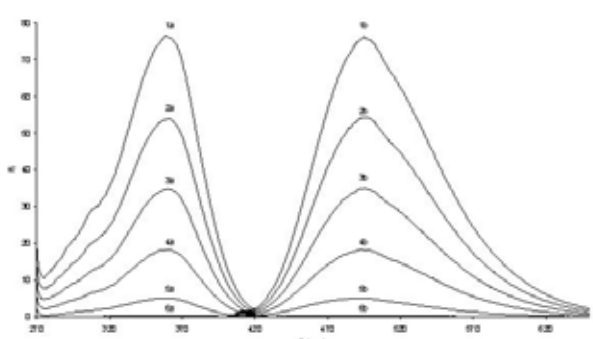

Fig. 1. **Excitation(a) and emission(b) spectra of Aluminium(III) in the presence of 7.4×10^{-5} mol dm^{-3} QSA at 620 V in dependence on concentration of Al(III) at pH 4**
1 – 1.6 μg cm^{-3}, 2 – 0.8 μg cm^{-3}, 3 – 0.4 μg cm^{-3}, 4 – 0.2 μg cm^{-3}, 5 – 0.05 μg cm^{-3}, 6 – 0 μg cm^{-3}

at pH 4 for Al^{3+}, $c_L = 1.5 \times 10^{-5}$ M at pH 3 for Ga^{3+} and $c_L = 3.5 \times 10^{-5}$ M at pH 8 for In^{3+} (Figs. 1. and 2.).

No fluorescence was observed for the sole reagent in this pH interval.

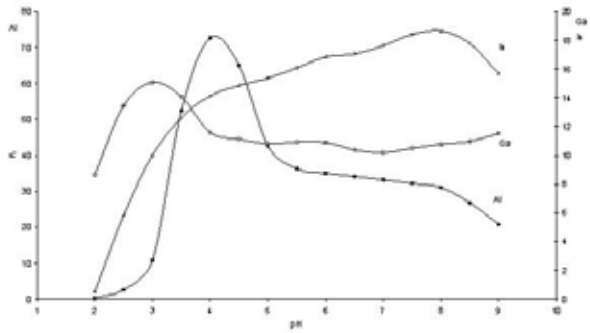

Fig. 2. **Fluorescence intensity dependence on pH for each complex (0.3 μg cm^{-3} of each element) at optimal conditions**

The first derivations of the fluorescence and excitation spectra under the same conditions as the normal spectra with $\lambda_{max(em)} = 460$ nm (AlQSA), $\lambda_{max(em)} = 472$ nm (GaQSA) and $\lambda_{max(em)} = 478$ nm (InQSA)are given in Fig. 3. Higher derivations could not be used because of a big noise of the instrument.

The calibration plots are stricly linear in solution with $c_L = 7.4 \times 10^{-5}$ M for Al^{3+} at pH 4 or $c_L = 5.8 \times 10^{-5}$ M QSA for Ga^{3+} at pH 3 and $c_L = 1.78 \times 10^{-5}$ M and pH 8 for In^{3+}.

Although the calibration plots are strictly linear till 2 μg cm^{-3}, the meaningful metal concentration range was 0.04–1 μg cm^{-3}. The decrease of QSA concentration in solution negatively influence the extent of the linear part of the calibration plot, the increase of QSA concentration causes quenching.

There is no considerable difference in the values of detection limits for normal spectra, derivative spectra and values in the presence of 0.0012 M cationic surfactant Zephyramine

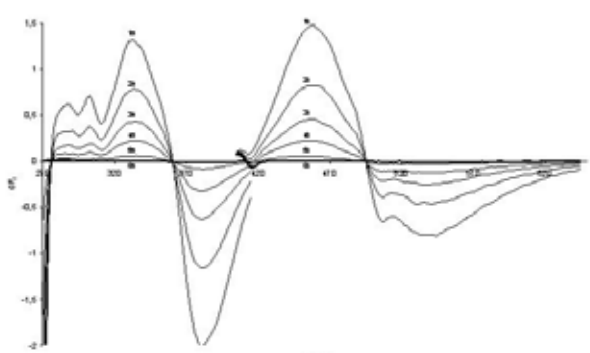

Fig. 3. **The first derivative excitation(a) and emission(b) spectra of Aluminium(III) in the presence of 7.4×10^{-5} mol dm^{-3} QSA at 620 V in dependence on concentration of Al(III).**
1 – 1.6 μg cm^{-3}, 2 – 0.8 μg cm^{-3}, 3 – 0.4 μg cm^{-3}, 4 – 0.2 μg cm^{-3}, 5 – 0.05 μg cm^{-3}, 6 – 0 μg cm^{-3}

when values according to Graham and IUPAC recommendation are compared.

Cationic surfactants increase considerably the intensity of fluorescence with no λ_{max} shift, but some little bathochromic shift (lesser than 20 nm) of excitation maximum is observed. The fluorescence increase is time dependent and takes 1 hour either during radiation or when the sample is left in darkness. Since the largest effect of the cationic surfactant is often observed for the CMC the formation of a fluorescent ion associate is assumed with the anionic metal complexes. The fluorescence, however, slowly decreases after reaching the micellar concentration of the surfactant.

The largest positive effect was found for 0.0012 M Zephyramine but for the subsequent increase of concentration a considerable decrease of fluorescence is observed for all cationic surfactants.

The anionic surfactants such as dodecylsulphate have a negative influence with the increasing concentration. No effect is observed for selected non ionic surfactants such as Triton X 100 and Brij 35, and β-cyclodextrine.

The effect of various surfactants follows from the Fig. 4. which shows relative fluorescence intensity dependence on surfactant concentration for GaQSA. The influence of surfactants for the AlQSA and InQSA is similar to that of GaQSA complex.

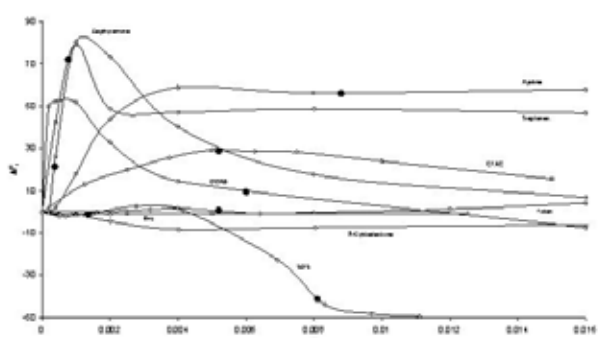

Fig. 4. **Surfactants influence on the GaQSA complex at pH 3. Septonex, Zephyramine, Ajatine, SDS – mol dm^{-3}, CTAC (Hexadecyl trimethyl ammonium chloride – c/4 [mol dm^{-3}], DDAB (Didodecyldimethyl ammonium bromide), β-Cyklodextrine – c/5 [mol dm^{-3}], Triton X 100 – c/4 × 10^{-5} [ppm], Brij – c/15 [mol dm^{-3}].**
Critical micellar concentrations are highlighted by the fulfilled marks

The fluorimetric method of continuous variations for at least two concentrations c_o gives unambiguously the simple mole ratio M : L = 1 : 1 in the Al^{3+}, Ga^{3+} and In^{3+} complexes at pH 3–4. For the increasing pH 8 and In^{3+} a higher complex is also indicated (Fig. 5.).

In the presence of constant concentration 0.0012 M of cationic surfactant Zephyramine a ratio M : L = 1 : 3 appears which may indicate the formation of a ternary species, $ML_3^{3-}.3\ T^+$.

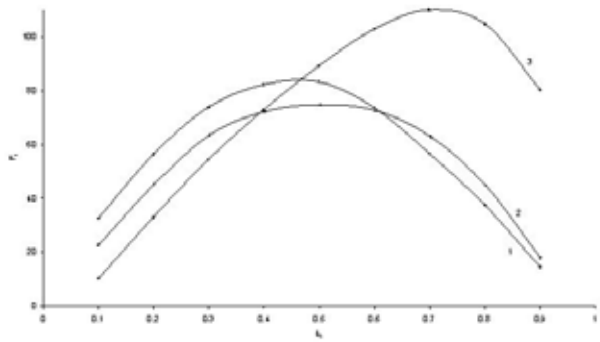

Fig. 5. **Continuous variations of AlQSA complex at pH 4. 1 – $c_0 = 1.1 \times 10^{-4}$ M, 635 V, 2 – $c_0 = 5.6 \times 10^{-5}$ M, 705 V, 3 – 5.6×10^{-5} M, 0.0012 M Zephyramine, 660 V**

There are no interferences observed for Na^+ and K^+ at 1,000 : 1 molar ratio. The effects of some other ions at optimalized conditions are summarized in Fig. 6. No considerable difference in the interferences is observed for the absence or presence of cationic surfactant Zephyramine (Fig. 6.).

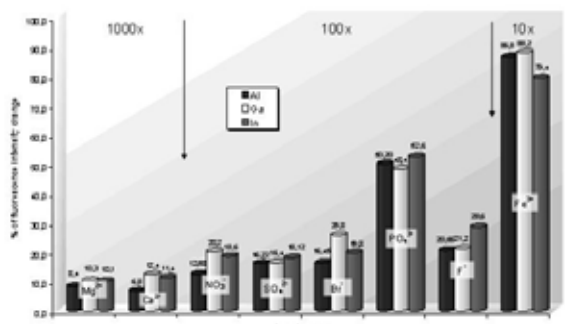

Fig. 6. **Interferences for 0.3 μg cm^{-3} of each element at optimal conditions in the presence of 0.0012 M Zephyramine**

For the application, 0.04–1 μg cm^{-3} of Al^{3+}, Ga^{3+} and In^{3+} are determined in the excess of QSA in the presence of 0.0012 M Zephyramine and 0.05 M hexamethylentetramine at pH 4 for Al, pH 3 for Ga and in the presence of 0.05 M tetraborate at pH 8. In the presence of 0.05 M hexamethylentetramine or sodium tetraborate 10% lower fluorescence intensity is observed. 0.05 M MES is not suitable because of larger fluorescence intensity decrease about 15 %.

Conclusions

The collinear fluorescence spectra of complexes with 8-Hydroxyquinoline-5-sulphonic acid were used for the study of Al, Ga, In complexes and determination of these elements at pH 4, 3 or 8. Strictly linear calibration plots for 0.04 – 1 μg cm^{-3} of the metal ion are observed under optimized conditions for the normal and first derivative spectra or in the presence of cationic surfactant.

The efficiency of fluorescence intensity increases in the presence of submicellar concentrations of cationic surfactants, especially Zephyramine. The detection limits from normal or first derivative spectra and in the submicellar media are practically identical (0.03–0.05 μg cm^{-3}), but sensitivity is significantly increased in the presence of Zephyramine. Fatal interferences are observed from Fe^{3+} and PO_4^{3-} only.

For the individual determination of Al, Ga and In with QSA hexamethylentetramine or sodium tetraborite buffers are suitable. The multicomponent determination of these ions can be carried out by approaching the PLS method[10].

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P88 SURGICAL POLYESTER FABRIC IMPREGNATED BY CROSS-LINKED COLLAGEN

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Introduction

Collagen is a widely applicable protein in medical applications. The main advantage of this biomaterial is its ability to create fibers with a high strength and stability by using different cross-linking agents or physical methods. Collagen's significant properties are biocompatibility and resorbability in organism, which might be particularly used at surface modification of surgical fabrics based on polyester silk (PES). The collagen modified PES nets used in surgery for longtime fixation or reinforcing different organs may accelerate healing injury as well as reduce inflammation of a tissue surrounding the implanted fabrics[1].

Natural crosslinking gives collagen special properties, namely higher rigidity and endurance against proteolytical cleavage. However, during processing and utilization the collagen loses those particular properties. That is why the collagenous material is additionally cross-linked by chemical or physical methods in order to regenerate original net behaviours.

Chemical methods employ bifunctional compounds (e.g. aldehydes, epoxides, isocyanides, carbodimides, acrylic acid etc.) which can react with amino groups of collagen in two different places resulting in generation of a new strengthing bond. Their main disadvantage is toxicity of used chemicals[2]. Instead of toxic aldehydes it might be used nontoxic 1-ethyl-3-(-dimethylaminopropyl) carbodiimide (EDC), which is soluble in water. For reaction acceleration the N-hydroxysuccinimide (NHS) as catalyst can be added to the system with EDC[3].

In this work cross-linking agents based on melamin-formaldehyde resin (named as LYOFIX and MH – 83) as well as carbodiimide (system EDC/NHS) were used for crosss-linking collagen impregnated on the polyester surgical net CHS 100. Swelling behaviours in the water and degradation at 37 °C in physiological solution with the purpose of increasing biocompatibility of commercial surgical net were examined.

Experimental

Modified derivative of alkylmelamineformaldehyde in aqueous solution (LYOFIX, Ciba, Hungary), hexamethylol-melamineformaldehyde resin (MH-83, Draslovka a.s. Kolin, CZ), 1-ethyl-3-3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, Sigma-Aldrich s.r.o.), N- hydroxysukcinimide (NHS 98 %, Sigma-Aldrich s.r.o.), $Na_2HPO_4 \cdot 12H_2O$ (Lach-Ner a.s. CZ), collagen type I (8 %, VÚP a.s., Brno), net for surgical purposes (CHS 100 – knitwork made from polyester silk, VUP a.s., Brno), ultra - clean demineralize and deionize MILIQ water (prepared in arrangement of Millipore S.A. at FCH BUT, CZ).

P r e p a r a t i o n o f a C o l l a g e n F i l m s

Polyester fabric CHS 100 was cut into same size square pieces of about 1 cm^2 which were diped layed on the 5 ml of 1% collagen solution (prepared from 100% freeze – dried collagen) embedded with extra 5 ml of 1% collagen solution. This sample was air-dried for approximately 48 h to the constant weight.

C r o s s - L i n k i n g

Collagenous films were cross-linked by 2% solution of LYOFIX and MN-83 resins for a period of 12 mins. Subsequently the samples were five times washed by the distilled water for 5 mins and dryied by air to the constant weight.

Collagenous films were cross-linked by the ethanol solution containing 50 mmol dm^{-3} of EDC and 25 mmol dm^{-3} of NHS. After 4 h of cross-linking the samples were washed out for 2 h in 0.1M solution of $Na_2HPO_4 \cdot 12H_2O$ and finally four times for half an hour in the distilled water followed by air-drying to the constant weight.

S w e l l i n g

Swelling characteristics were evaluated to compare the cross-linking effectivity by each agent. Swelling proceeded for a period of 40 min to 1 h in MILIQ water at a laboratory temperature. Quantity of absorbed water was weighted in five minute intervals. Both water content (OV) and swelling ratio (SB) of each sample were calculated according to equation (1) and (2), where m_C is weight of swollen sample in a given time and m_S is weight of dry sample prior the swelling[4].

$$\mathrm{OV}\,[\%] = \frac{m_C - m_S}{m_C} \cdot 100 \qquad (1)$$

$$\mathrm{SB}\,[-] = \frac{m_C}{m_S} \qquad (2)$$

D e g r a d a t i o n

Each sample was placed in the physiological solution with addition of sodium azide (0.1% NaCl + 0.02% Na_3N) and placed into the incubator set to 37 °C to determine the hydrolytic degradation. Prepared samples were every day taken out from the incubator, dried by the filtration paper, consequently weighted and immediately placed back with fresh physiological solution. Degradation was expressed as percentual decrease of collagen weight in certain time having the zero point at the maximum of swelling according to equations (3) and additionaly (4)–(6).

$$\text{Degradation} = 100 - ((\text{Wk} \cdot 100)/\text{Wb}) \quad (3)$$

$$\text{Wk} = \text{W} - \text{W}_{sb} \quad (4)$$

$$\text{Wsb} = \text{W}_{ss} + \text{W}_{ss} \cdot \text{OV}_{13} \quad (5)$$

$$\text{OV}_{13} = \frac{W_{sb} - W_{ss}}{W_{sb}} \cdot 100 \quad (6)$$

W_k represents weight of swollen collagenous sample without wet net, W_b is weight of swollen collagenous sample at maximum of swelling, W_{sb} is weight of wet net, W_{ss} is weight of dry net, W is weight of swollen collagenous sample with net and OV_{13} is water in net after 13 days of swelling (%).

M o r p h o l o g y

Morphology of samples was observed by the scanning electron microscope Philips Quanta 200. On the sample surface conductive lay of 3–4 nm were steamed by the sputter Polaron SC7640 prior the analysis.

Result and Discussion

S w e l l i n g R a t i o

Swelling ratio (SB) of all prepared samples shows Fig. 1. It can be seen that the highest swelling ratio attained non cross-linked collagen (SB = 42 in 25 min), then non cross-linked collagen coated on the net (SB = 14 in 25 min). Cross-linked collagens have almost same swelling ratio (SB = 3–4 in 25 min). The lower amount of water absorbed polyester net (SB = 1 in 25 mine).

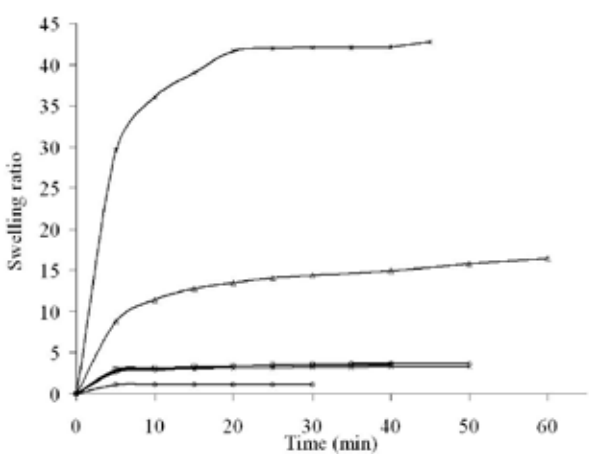


Fig. 1. **Dependence of swelling ratio on time for samples cross-linked by the EDC – NHS (◇), LYOFIX (□), MH-83 (×), non cross-linked collagen with net (△), non cross-linked collagen without the net (∗) and the net without collagen (○).**

D e g r a d a t i o n

From the degradation behavior dependence of collagen in the physiological solution at 37 °C on time (Fig. 2.) it is assumed that the fastest degradation yielded non cross-linked collagen within 21 days of incubation. After 23 days degraded non cross-linked collagen coupled to the net resulting in net maintain. The slowest degradation in the physiological solution showed the collagen cross-linked by EDC-NHS which recorded 59 % of degradation in 78 days of incubation. The shortest degradation of cross-linked collagen embodied sample treated by LYOFIX resin (17 % of degradation in 24 days) followed by MH-83 resin (32 % of degradation in 35 days).

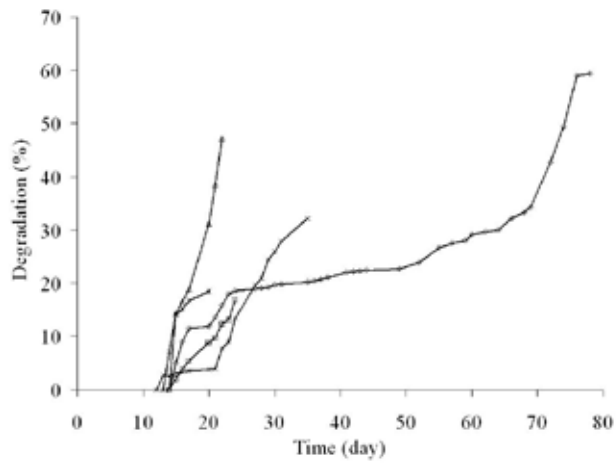
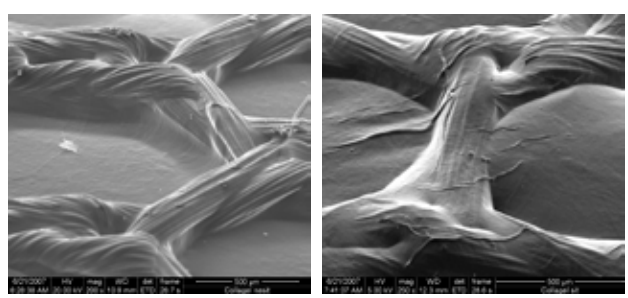


Fig. 2. **Dependence of degradation on time of collagenous samples coss-linked by the EDC – NHS (◇), LYOFIX (□), MH 83 (×), non cross-linked collagen on the net (△), non cross-linked collagen without the net (∗)**

M o r p h o l o g y

Scanning electron microscope picture in Fig. 3.a (sight angle of 54 °) shows good adhesion between the non cross-linked collagen coated on the PES net as well as between the collagen cross-linked by the EDC-NHS and the PES fabric (Fig. 3.b).

Cross-linked samples after the degradation were observed in environmental mode. From the Fig. 4. it is evident the separation of collagen from the polyester net in this case



(a) (b)

Fig. 3. **Morphology of collagen built – up on the net CHS100 non cross-linked (a) and cross-linked by the EDC – NHS (b)**

cross-linked by the EDC – NHS after the 78 days of incubation in the physiological solution.

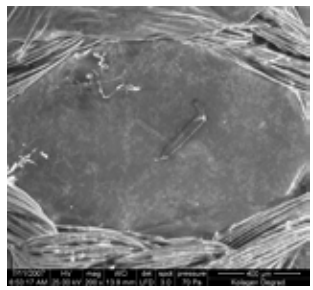


Fig. 4. **Morphology of degraded collagen coated on the net and cross-linked by the EDC – NHS**

Conclusions

The all cross-linked collagen samples embodied very good adhesion to the net when coated and did not show any separation until the total degradation. Samples cross-linked by the new system of EDC-NHS attained approximately the same swelling ratio like samples cross-linked by the resins of LYOFIX and MH-83. The biggest strength of polymeric net and hydrolytic degradation resistivity showed sample cross-linked by the EDC-NHS system, which were stable against the degradation for 78 days in physiological solution at 37 °C (confirmed by microscopy). Due to the obtained properties the new cross-linked collagen might enhance the biocompatibility of commercially used net CHS 100 since it is coated and implanted. Moreover, collagen itself might be modified (e.g. with hyaluronic acid) in order to speed up the wound healing process or reduce the tissue inflammation in the net surrounding environment.

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P89 SOIL HYGIENE IN OLD ENVIRONMENTAL BURDEN AREAS

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Introduction

The necessity of old environmental burden areas remedy is one of present environmental problems. The negative effect of the anthropogenic breach into the environment arised in the past still exists. It can be often a potential source of risk element input into the all components of the environment. The old scrap-heaps, mines, landfills, industrial, biological[1] and chemical wastes, heavy metals[2] and oil products are important factors of the environment contamination.

In our research work the soil hygiene of three various old environmental burden areas of Slovakia is evaluated.

The first one is the wider surrounding of previous Nickel smeltery in Sereď situated in the loaded region Dolný Váh, one of 12 regions of the Slovak Republic with the most contaminated soils by risk elements[3]. The second observed region is the alluvial area near of Štiavnica river running through the area long-time burden by intensive mine activity[4]. The third surveyed locality is the wider surrounding of Iron ore mines in Rudňany. This enterprise belonged in the past to the one of determining sources of the emission contamination of the environment in loaded area of Stredný Spiš.

The risk of heavy metal input from residual metallic burden soil into the food plants as well as into the whole human food chain is in observed regions evident.

Experimental

The observation of risky elements in soils were realized on parcels in cadasters of villages: Zavar, Križovany, Vlčkovce, Hoste, Malá Mača and Veľká Mača in the distance of 5 to 9 km in northern and nort-western direction and in cadasters of villages Veľká Mača, Šintava and Vinohrady nad Váhom in the distance of 2 km south-western, 2.8 km eastern and 3.5 km north-eastern from former emmission source of Nickel smeltery Sereď.

In longitudinal north-southern direction of river Štiavnica alluvium 11 soil samples from cadastres of villages Prenčov, Hontianske Nemce, Hontianske Tesáre, Terany, Dudince, Hokovce, Horné Semerovce, Tupá, and Hrkovce were taken. The river length is 54.6 km. The distance between Prenčov and Hrkovce is about 36 km.

In the Stredný Spiš region the soil samples were taken in cadaster of Markušovce in the distance of 10–12.5 km north-western, Matejovce 7.6 km northern, Chrasť nad Hronom 10 km north-eastern and Poráč 6 km eastern of former emmission source ŽB Rudňany.

The samples with pedological probe from surface soil layer were taken from 33 experimental sites. Analyses were conducted in samples of soil ground on fine soil I. and from this fine soil the representative sample was taken and sieved through the sieve with average 0.2 mm (fine soil II). The total content of risky elements was determined by the AAS method in soil extract gained after total decomposition of soil by wet way with the mixture of acids HF + HNO_3 + $HClO_4$.

Results

The soil hygiene was evaluated after Resolution of the Ministry of Agriculture of the Slovak Republic No. 531/1994-540, which determines the hygienic limits for soil contents of 15 selected risk elements. The reference value A means that the soil is not contaminated if the substance concentration is below this value, the indicative value B means that soil contamination was analytically proven, the indicative value C means the necessity of the soil sanitation. The soils surround of previous Nickel Smeltery Sereď could be considerated from the standpoint of risky metals contents as relatively „clean“, in spite of moderately enhanced contents of Cd, Cu, Ni and Co. There was no content value meaning analytical proof of soil contamination. The Cd content was in the range from 1.6 to 2.5-fold of background value A (0.8 mg Cd kg^{-1}) in all observed parcels. The Ni content exceeded the value A (35 mg Ni kg^{-1}) in all parcels with the exception of Vinohrady nad Váhom locality, while it was enhanced from 1.1 to 1.4-fold. The Cu content was increased in all localities with the exception of Zavar and Veľká Mača (1.03 to 1.93-fold of value A 36 mg Cu kg^{-1}). The enhanced soil content of Co was observed in Zavar, Hoste, Veľká Mača and Šintava (1.06 to 1.22-fold of value A 20 mg Co kg^{-1}). All of the determined values were deeply under the hygienic limit for the soil contamination.

In soils of the second observed region of alluvium Štiavnica river the soil contamination by high contents of Cu, Zn, Cd and Pb was confirmed. The determined Pb contents were extremely high in all of analysed soil samples and they even markedly exceeded the indicative limit value for soil sanitation C (600 mg Pb kg^{-1}) given by valid legislative.The Cu and Pb contents in observed soils were the highest in Prenčov (2.6-fold of indicative value B 100 mg Cu kg^{-1} resp. 5.4-fold of indicative value C for Pb), the highest Zn and Cd contents were determined in soil of Hontianske Nemce (5.52-fold resp. 4.24-fold of indicative values B 500 mg Zn kg^{-1} resp. 5 mg Cd kg^{-1}). The lowest Cu soil content was determined in Horné Semerovce (on the level of the indicative value B), the lowest Zn, Cd and Pb contents were determined in Hontianske Tesáre (Zn on the level of the limit value B, Cd 30% under the limit and Pb 1.78-fold of the indicate value B 150 mg Pb kg^{-1}).

The situation in the wider surrounding of the third surveyed site of the former emmission source was different. The enormly high content of Hg was found out in Markušovce

in locality Olšanské pole, which was even exceeding the hygienic limit determining the soil sanitation (1.9 to 3.8-fold of value C 10 mg Hg kg^{-1}). Similarly the extremely high content of Hg was determined in Poráč, where the 3.2-fold higher exceeding of this limit value was determined. The contamination of soil by Hg was obvious also in localities: Pod horky, Zemkovské (Markušovce) and Na stráni (Matejovce because of the exceeding of limit value B (2 mg Hg kg^{-1}) for the soil contamination at 4.65-fold. Similarly the soil contamination by As was proved in Markušovce (locality Olšanské pole), where the limit value B (30 mg As kg^{-1}) was exceeded 1.45-fold and Poráč (locality Pasienky) with 1.06-fold In Poráč the soil contamination by Cu was evident (1.15-fold of limit B).

Conclusions

The residual soil burden by heavy metals and potential follow contamination of the food chain in observed areas presents the potential risk for the human health. Therefore it is important to monitor the risk metal contents as in soils as in agricultural plants and in case that it is necessary to realise the remedy for the inhibition of the risk heavy metal input into the human food chain.

This work has been supported by projects KEGA No. 3/5081/07 and VEGA No. 1/3455/06

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P90 EPR STUDY ON PHOTOINDUCED PROCESSES OF NOVEL QUINOLONE DERIVATIVES

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Introduction

4-Oxo-1,4-dihydroquinoline derivatives (4-quinolones) represent one of the largest classes of antimicrobial agents used nowadays in the medical care[1]. Specific members of this drug family display also high activity against eukaryotic type II topoisomerases, as well as against cultured mammalian cells[2]. These antineoplastic quinolones represent a prospective source of new anticancer agents. The presence of extended π-electron system of quinolone derivatives results in their photosensitive properties; therefore UVA irradiation can induce their photosensitive reactions with phototoxic responses. Previously we demonstrated that excitation of nitrogen heterocycle molecules by UVA radiation may significantly enhance their biological activities[3].

Novel 7-substituted 6-oxo-6,9-dihydro[1,2,5]selenadiaz o-lo[3,4-*h*]quinoline derivatives were synthesized as potential anticancer and antimicrobial agents and their ability to produce Reactive Oxygen Species (ROS) upon irradiation was tested using Electron Paramagnetic Resonance (EPR) spectroscopy. Additionally, their cytotoxic/phototoxic effects on human leukemia cells HL60 were characterized.

Experimental

The synthesis of 7-substituted 6-oxo-6,9-dihydro[1,2,5]-selenadiazolo[3,4-*h*]quinoline derivatives was performed according to the reaction pathways published in ref.[4].

Table I summarizes the structure, substitution and abbreviation of the synthesized selenadiazoloquinolones.

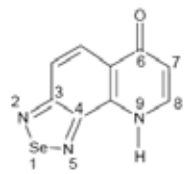
E P R P h o t o c h e m i c a l E x p e r i m e n t s

EPR measurements at the X-band were performed with an EMX EPR spectrometer (Bruker, Germany) using a TE_{102} (ER 4102ST) resonator. Samples were irradiated at 295 K directly in the EPR spectrometer cavity using HPA 400/30S lamp (400 W, l_{max} = 365 nm, Philips, UVA irradiance 3 mW cm^{-2}). A Pyrex glass filter was applied to eliminate the radiation wavelengths below 300 nm. The experimental EPR spectra acquisition and simulation was carried out using *WIN EPR* and *SimFonia* programs (Bruker).

The photoinduced generation of free radicals was monitored by spin trapping technique with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 5-(diisopropoxyphosphoryl)-5-methyl-1-pyrroline N-oxide (DIPPMPO) as the spin traps. The selective oxidation of 4-hydroxy-2,2,6,6-tetramethylpiperidine (TMP) *via* singlet oxygen to the paramagnetic nitroxyl radical 4-hydroxy-2,2,6,6-tetramethylpiperidine N-oxyl (Tempol) was applied for $^1O_2$ detection by EPR spectroscopy. The aerated solutions of 7-substituted [1,2,5]selenadiazolo[3,4-*h*]quinolo-nes in dimethylsulphoxide (DMSO) mixed immediately before EPR experiments, were transferred into a flat cell (WG-808-Q, Wilmad) suitable for TE_{102} cavity, and the EPR spectra were monitored *in situ*.

UV/visible spectra were recorded in DMSO by means of a Shimadzu UV-3600 spectrophotometer.

Table I
Structure, substitution and abbreviation of investigated 7-substituted 6-oxo-6,9-dihydro[1,2,5]-selenadia-zolo[3,4-*h*]quinoline derivatives

Structure	7-Substitution	Abbreviation
	H	7-H-SeQ
	$COOC_2H_5$	7-COOEt-SeQ
	$COOCH_3$	7-COOMe-SeQ
	COOH	7-COOH-SeQ
	$COCH_3$	7-Ac-SeQ
	CN	7-CN-SeQ

C y t o t o x i c / P h o t o t o x i c E f f e c t

The murine leukemia cell line HL60 (obtained from American Type Culture Collection, Rockville, MD, USA) was used. These cells were grown in RPMI medium in 5 % CO_2 at 37 °C under conditions specified in ref.[4]. A starting inoculum 2.6×10^5 HL60 cells ml^{-1} in the exponential phase of growth was used. 5 ml of the suspension were added into Petri dishes (diameter 60 mm), then 20 µl of derivative 7-Ac-SeQ at various concentrations were added to the cells. One part of the dishes was irradiated with HPA 400/30S lamp upon UVA dose of 0.4 J cm^{-2}. After 24, 48 and 72 h cultivation, the number of cells per culture dishes was counted in a Bürker chamber and viability of treated and control irradiated/non irradiated cells were determined by 0.4 % trypan blue staining.

Results

7-Substituted 6-oxo-6,9-dihydro[1,2,5]selenadiazolo[3,4-*h*]quinoline derivatives absorb UV radiation with three absorption maxima about 400, 340 and 300 nm, which are only slightly influenced by the substituents properties, as is shown in Fig. 1. The presence of acetyl substituent in 7-Ac-SeQ derivative caused bathochromic shift of the low-energy band to 410 nm with a shoulder at 485 nm.

Upon photoexcitation, all 7-substituted 6-oxo-6,9-dihy dro[1,2,5]selenadiazolo[3,4-*h*]quinoline derivatives demonstrated the ability to generate super-oxide anion radicals trapped as $^{\bullet}$DMPO-O_2^- or $^{\bullet}$DIPPMPO-O_2^- spin adducts.

Fig. 2.a shows the time-course of 11 individual EPR spectra monitored upon continuous UVA irradiation of 7-COOEt-SeQ, indicating the efficient generation of twelve-line EPR signal with spin Hamiltonian parameters corresponding to $^{\bullet}$DMPO-O_2^- (a_N = 1.275 mT; a_H^{β} = 1.032 mT;

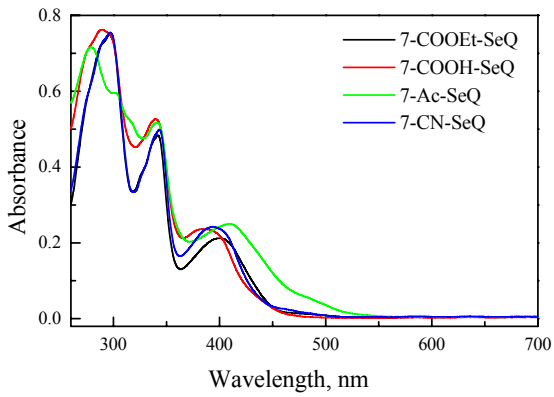


Fig. 1. **UV/VIS spectra of 7-substituted 6-oxo-6,9-dihydro[1,2,5]selenadiazolo[3,4-h]quinoline derivatives measured in DMSO (concentration 40 μM; cell length 1 cm)**

a_H^{γ} = 0.135 mT and g-value = 2.0058). Additionally, Fig. 2. b illustrates the formation of typical three-line EPR signal of Tempol (a_N = 1.575 mT; g = 2.0060) produced from TMP *via* singlet oxygen. However, photogenerated paramagnetic Tempol is upon prolonged irradiation decomposed to diamagnetic products, most probably by the termination of its nitroxyl group with super-oxide anion radicals (>NO$^{\bullet}$ + $O_2^{\bullet-}$).

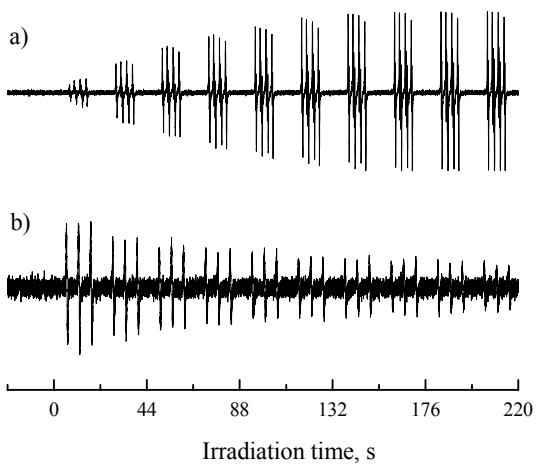


Fig. 2. **The time-course of EPR spectra monitored upon photoexcitation of aerated DMSO solutions of 7-COOEt-SeQ (c_0 = 3.2 mM) in the presence of: a) DMPO spin trap (magnetic field sweep, SW = 10 mT); b) TMP (SW = 6 mT)**

The EPR experiments confirmed that the photoexcitation of selenadiazoloquinolones in the presence of molecular oxygen resulted in the formation of $O_2^{\bullet-}$ and $^1O_2$, and these processes perform simultaneously. The photoactivity of 7-sub-stituted 6-oxo-6,9-dihydro[1,2,5]selenadiazolo[3,4-*h*]quinoline derivatives upon irradiation of polychromatic UV source was evaluated using *(i)* quantum efficiency of spin-adduct formation of superoxide radical with DMPO spin trap ($^{\bullet}$DMPO-O_2^-), and *(ii)* quantum efficiency of Tempol generation from TMP by singlet oxygen. The values of quantum efficiencies reflect the donor/acceptor properties of substituents; the highest value of $^{\bullet}$DMPO-O_2^- quantum efficiency was found for 7-acetyl 6-oxo-6,9-dihydro[1,2,5]selen adiazolo[3,4-*h*]quinoline (7-Ac-SeQ).

The *in vitro* cytotoxic effect of six 7-substituted 6-oxo-6,9-dihydro[1,2,5]selenadiazolo[3,4-*h*]quinoline derivatives was investigated on cell proliferation of human leukemia HL60 cells. Derivatives demonstrated different cytotoxic effects, which were time- and concentration- dependent, and the highest impact was found for 7-Ac-SeQ. The application of UVA irradiation caused an escalation of 7-Ac-SeQ effects on cell proliferation; the percentage of growth inhibition was increased in the range of 10–50 %.

Conclusions

EPR and UV/Vis experiments confirmed UVA-induced excitation of 7-substituted 6-oxo-6,9-dihydro[1,2,5]selenadiazolo[3,4-*h*]quinoline derivatives, which is coupled with electron or energy transfer to molecular oxygen *via* Type I and Type II photooxidation mechanisms producing super-oxide anion radical and singlet oxygen.

Dedicated to Prof. Andrej Staško on the occasion of his 70th birthday.

This study was financially supported by Scientific Grant Agency of the Ministry of Education of the Slovak Republic (Projects VEGA 1/4305/07, 1/0225/08 and VEGA 1/3579/06) and Research and Development Agency of the Slovak Republic (contract No. APVV 0055-07).

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P91 THE BORON IN KRAFT PULP MILL AND INFLUENCE IN WASTE WATER

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Introduction

The elemental boron almost is not occurred in nature and it usually is component only its different compounds. The boron forms boranes with hydrogen. It forms acids of these types: $(HBO_2)_n$ and H_3BO_3. The borides are boron compounds with metals (Me) and boron forms BX_3 with halides (X_3). The oxycompounds are formed with boron and oxygen, i.e. B_2O_3 and $(BO)_n$. B_2O_3(l) [molten] dissolves oxides of metals (borax pearls). Wide range of boron compounds exists with different stoichiometry and structure (see Fig. 1.). The boron more often exists as borax in nature. The molten borax covers molten other metal and so it is protective compound against oxidation. The mixture boron and Na_2CO_3 is used for dissociation of geological and hard dissolving samples[1,2].

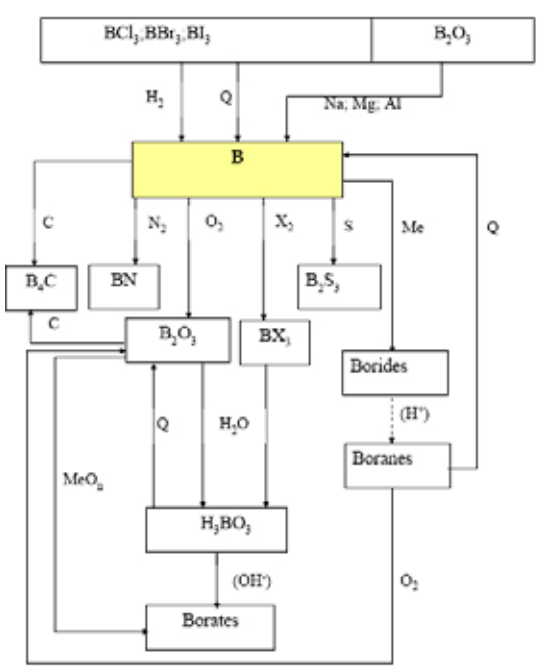

Fig. 1. **Chart for Boron reaction**

Borax is used in recovery line for process improvement in kraft pulp mill too. The conventional recovery cycle with addition of borates; this produces 10 % of the formed NaOH (partial autocaustification). The borates react in two places in the recovery cycle: the recovery boiler and the smelt dissolver. In the former sodium carbonate reacts with a sodium-lean borate, forming a sodium-rich borate formed: $NaBO_2(l) + Na_2CO_3(l) = Na_3BO_3(l) + CO_2(g)$. In this reaction, the borates and the sodium carbonates are in a smelt phase, which implies that the reaction rate is high. In the dissolver, Na_3BO_3 reacts with water: $Na_3BO_3(l) + H_2O =$ 2NaO H(aq) + $NaBO_2$(aq)(ref.[3]).

Fig. 2. **Borax $Na_2B_4O_7 \cdot 10H_2O$ i.e. Sodium tetraborate. It is applied in different industry: metallurgy, glass industry, ceramic industry and in some pulp mill**

Boron compounds are occurred in fresh/salt water and in waste water too. Concentration of boron compounds is app. 5 mg dm^{-3} in salt water[4]. Boron occurs naturally in fresh water at an average of 1 mg dm^{-3} or less many times. High boron concentrations may be toxic to freshwater fish regarding concentration of 10–300 mg dm^{-3} (e.g. for Rainbow trout 24-day LC50 is 88 mg dm^{-3}) in flowing water. Mainly borate is hazardous for water plants (e.g. for Green algae 96-hr IC10 is 24 mg dm^{-3}). Any definitive ecotoxicological conclusions don't exist about genotoxic, carcinogenic or mutagenic effects to water organisms, but still under investigation[5].

Experimental

The autocaustification was realized in kraft pulp mill. Trial was started on December 1, 2007 till December 14, 2007 and restarted again on January 4, 2008. This trial was finished on February 25, 2008. Waste water was monitored for boron concentrations in two different laboratories. The boron concentration was determined in the first laboratory (spectrophotometry with azomethin at 415 nm) as calculation from borates. The boron also was analyzed using OES ICP method (in 2nd laboratory) the first time in different samples of waste water, which were from different pH and COD. Alkali waste water, acid waste water, waste water of sewage water treatment plant, water from river and output to river were analyzed for boron in two steps, i.e. during trial and after trial in 2nd laboratory with OES ICP.

Borax was added into dissolvent tank for green liquor step by step. Boron concentration was increased in waste water in February, when borax was added more in recovery line.

Results

Boron analysis was difficult because of small particulates, which were filtrated from samples, but boron concentration was estimated from samples, which were not filtrated.

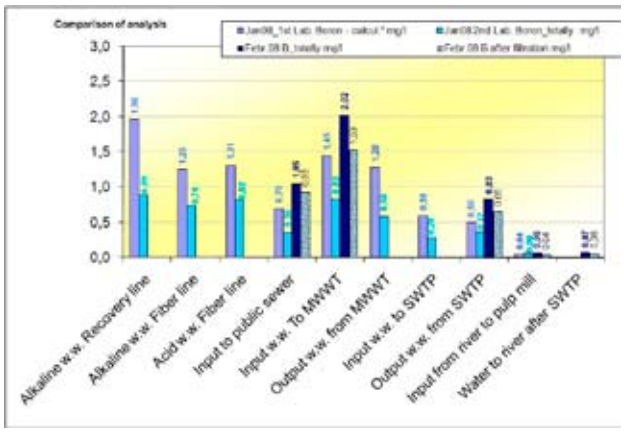

Fig 3. **Comparison of B analysis**
Note : w.w. = waste water, MWWT = mechanical waste water treatment, SWTP = sewage water treatment plant

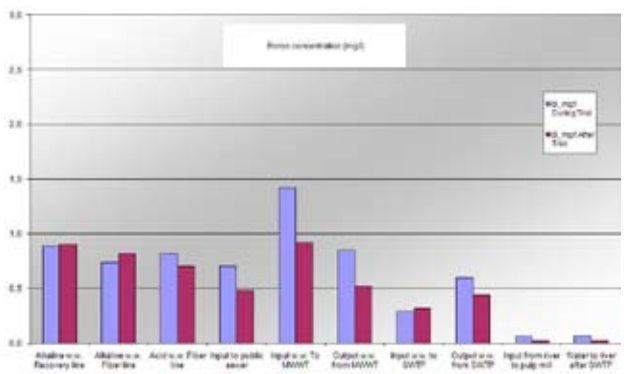

Fig. 4. **Boron concentrations**

The results are from these samples, because boron could be component in small particulates of waste water samples. Boron concentration is low in waster water which is cleaned in SWTP. The limits are not specified by low for boron concentration in waste water to the water recipient.

In the Danube, water (Komárno – Bratislava) was determinate to have a natural concentration on the level of 39 $\mu g\,dm^{-3}$(ref.[6]) and for river is the level of 20 $\mu g\,dm^{-3}$ in the river after the biological waste water treatment plant and 20 $\mu g\,dm^{-3}$ in recipient to pulp mill. Boron concentration was low in lime sludge too. Boron was accumulation in system and its output was waste water, but boron concentration was low.

Conclusions

In this work was studied effect of adding boron compound to recovery line on the water quality.

- Boron is component in very small particulates in waste water, therefore boron concentration is better without filtration of samples for boron determination. Boron concentration is not total in waste water by filtration.
- Boron concentration is low in output - waste water to river, what corresponding accumulation of boron compounds in system of pulp mill.
- There was little decreasing of boron concentration after trial, but boron compounds were in system.

Open question:

- What about deposits in recovery line and pipeline of waste water?

This work has been supported by Kraft pulp mill.

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P92 CONTENTS OF DIFFERENT FRACTIONS OF SULPHUR IN SLOVAKIA SOILS

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Introduction

In the past there were high sulphur contents in the soil, mostly from the atmospheric inputs. Nowadays, in some localities deficit of sulphur was observed. In the last time advanced quality of environment is required by society. The aim of this work was to identify the distribution of sulphur fractions in different soil types of Slovakia.

Experimental

Basic sulphur fractions that we assessed in soil samples were: chloride soluble sulphur (CISS), sulphate sulphur (SS), heat soluble sulphur (HSS) by Williams-Steinbergs. Soil samples were taken from similar depths from 52 localities including basic soil types: Haplic Chernozems (CHH), Haplic Luvisols (LVH), Phaeozems (PH), Eutric Cambisols (CME), Dystric Planosols (PLD), in 1999–2006 years. Analytical methods of sulphur fractions determination were described by Jedlovská, Feszterová[1].

Results and Discussion

Chloride Soluble Sulphur Fraction (ClSS)

The chloride soluble sulphur values (ClSS) were determined as extremely hight in Phaeozems, in localita Maňa, Žitavský luh 2,744.5 mg kg^{-1} (depth: 0–0.1 m) and in locality Okoč 1,173.6 mg kg^{-1} (0–0.4 m). Generally, the contents of ClSS varied with depth, the highest values were observed in subsoil layers. The minimum values were determined in vineyard on Eutric Cambisols 3.2 mg kg^{-1} (0.3–0.6 m; Tokaj) and in textural light Haplic Luvisols 4.4 mg kg^{-1} (0.5–0.6 m; Rišňovce).

Sulphate Sulphur Fraction (SS)

The highest sulphate sulphur content was in Haplic Luvisols locality Golianovo (1,191.7 mg kg^{-1}; depth: 0–0.2 m) and Nové Sady (856.1 mg kg^{-1}; 0.2–0.4 m). The content of sulphate sulphur varies by soil types and depth of soil profiles. The minimum values (from 1.2 mg kg^{-1} to 3.7 mg kg^{-1}) were observed in topsoil of Haplic Chernozems (locality Štefanovičova), Eutric Cambisols (locality Viničky) and Haplic Luvisols (Rišňovce).

Table I
Contents of chloride soluble sulphur (ClSS)

Soil types/ (numbers)	Depth [m]	Mean [mg kg^{-1}]	Standard deviation
Haplic Chernoyems (14)	0.0–0.2	192.5	175.2
	0.2–0.4	92.8	61.2
	0.4–0.6	151.0	132.5
	0.6–0.8	98.7	71.8
	0.8–1.4	70.0	66.1
Phaeozems (6)	0.0–0.2	234.0	151.4
	0.2–0.4	373.7	271.5
	0.4–0.6	358.0	220.4
	0.6–0.8	453.2	303.0
	0.8–1.4	261.7	81.8
Haplic Luvisols (17)	0.0–0.2	305.3	274.3
	0.2–0.4	299.9	259.8
	0.4–0.6	299.1	254.7
	0.6–0.8	375.3	333.6
	0.8–1.0	384.4	299.7
	1.0–1.2	395.5	275.0
	1.2–1.4	244.3	201.6
Dystric Planosols (5)	0.0–0.2	82.0	77.0
	0.2–0.4	45.4	32.5
	0.4–0.6	63.7	20.3
	0.6–0.8	106.2	72.4
	0.8–1.4	121.8	118.5
Eutric Cambisols (10)	0.0–0.2	130.0	125.9
	0.2–0.4	157.4	157.3
	0.4–0.6	159.2	154.3
	0.6–0.8	171.8	156.4
	0.8–1.4	187.7	115.0

Heat Soluble Sulphur Fraction (HSS)

The lowest values of HSS fraction were determined in comparison to the mentioned sulphur fractions. The highest HSS content was in Phaeozems locality Žitavský luh (1,837.4 mg kg^{-1}; depth: 0–0.1 m), in Haplic Luvisols locality Revúca (617.5 mg kg^{-1}; 0–0.3 m) and in Haplic Chernozems locality Sládkovičovo- Nový Dvor (433.8 mg kg^{-1}; 0.5–0.7 m). The minimum of HSS (from 0.28 mg kg^{-1} to 1.2 mg kg^{-1}) were observed in topsoil of Eutric Cambisols (Tokaj), of Haplic Luvisols (Tesárske Mlyňany) and in sandy loam Haplic Chernozems (Dulovce).

Conclusions

The contents of sulphur fractions (ClSS, SS, HSS) varied by depth of soil profiles and soil types. The maximumn values of ClSS fraction were observed in Phaeozems (336.1 mg kg^{-1}) and Haplic Luvisols (329.1 mg kg^{-1}). The values under average values of ClSS (206.2 mg kg^{-1}) were in Eutric Cambisols (161.2 mg kg^{-1}), Haplic Chernozems (121.0 mg kg^{-1}) and Dystric Planosols (83.8 mg kg^{-1}). The highest contents of SS fraction were determined in Haplic Chernozems

Table II
Contents of sulphate sulphur (SS)

Soil types/ (numbers)	Depth [m]	Mean [mg kg^{-1}]	Standard deviation
Haplic Chernozems (14)	0.0–0.2	162.1	134.2
	0.2–0.4	184.2	144.9
	0.4–0.6	116.9	97.9
	0.6–0.8	149.7	108.7
	0.8–1.4	120.9	87.0
Phaeozems (6)	0.0–0.2	213.2	125.7
	0.2–0.4	218.4	52.0
	0.4–0.6	263.2	140.7
	0.6–0.8	428.9	187.2
	0.8–1.4	377.3	145.5
Haplic Luvisols (17)	0.0–0.2	323.0	320.3
	0.2–0.4	389.5	234.6
	0.4–0.6	234.2	117.7
	0.6–0.8	242.0	124.3
	0.8–1.0	223.7	46.7
	1.0–1.2	281.4	64.6
	1.2–1.4	310.0	62.2
Dystric Planosols (5)	0.0–0.2	38.7	35.9
	0.2–0.4	53.5	64.3
	0.4–0.6	53.9	46.7
	0.6–0.8	50.3	36.8
	0.8–1.4	42.2	30.5
Eutric Cambisols (10)	0.0–0.2	92.4	79.0
	0.2–0.4	103.1	91.6
	0.4–0.6	101.2	87.3
	0.6–0.8	154.3	59.9
	0.8–1.4	125.0	82.8

Table III
Contents of heat soluble sulphur (HSS)

Soil types/ (numbers)	Depth [m]	Mean [mg kg^{-1}]	Standard deviation
Haplic Chernozems (14)	0.0–0.2	93.4	89.0
	0.2–0.4	143.8	115.8
	0.4–0.6	132.4	130.7
	0.6–0.8	110.4	89.1
	0.8–1.4	115.8	93.4
Phaeozems (6)	0.0–0.2	207.4	109.5
	0.2–0.4	249.8	92.7
	0.4–0.6	227.7	96.1
	0.6–0.8	213.3	36.2
	0.8–1.4	214.7	31.2
Haplic Luvisols (17)	0.0–0.2	145.3	106.7
	0.2–0.4	161.5	84.8
	0.4–0.6	152.9	86.8
	0.6–0.8	163.5	127.2
	0.8–1.0	146.9	51.8
	1.0–1.2	131.3	71.2
	1.2–1.4	127.7	48.1
Dystric Planosols (5)	0.0–0.2	53.5	52.5
	0.2–0.4	76.5	46.2
	0.4–0.6	77.1	24.3
	0.6–0.8	89.1	31.2
	0.8–1.4	56.8	32.0
Eutric Cambisols (10)	0.0–0.2	203.6	140.4
	0.2–0.4	130.0	134.6
	0.4–0.6	166.1	144.7
	0.6–0.8	239.8	173.7
	0.8–1.4	131.0	83.2

(300.2 mg kg^{-1}) and in Haplic Luvisols (286.3 mg kg^{-1}), while in Dystric Planosols the values were only 47.7 mg kg^{-1}.

The differences between soil types were determined in HSS fraction, where the highest mean values observed in Phaeozems and Eutric Cambisols. The lowest value of HSS fraction was in Dystric Planosols.

Decreasing contents of ClSS fraction, in monitoring of soils types where: PH > LVH > CH > CME > PLD, the decreasing contents of SS fraction was LVH > PH > CH > CME > PLD. Presented results show the possibilities how to evaluate the different fractions of sulphur in different soils types in Slovakia.

This work has been supported by grant VEGA 1/4432/07.

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P93 FACTORS INFLUENCING THE SORPTION BEHAVIOUR OF HERBICIDE ACETOCHLOR IN SOILS AND SEDIMENTS

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Introduction

Acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylfenyl)acetamide) is a selective systematic herbicide used in the production of maize, soybean and other crops[1]. Ability to predict its mobility in the environment is of major importance as US EPA has classified acetochlor as a probable human carcinogen[2].

The aim of this study was to investigate the sorption and desorption of acetochlor in various types of natural sorbents (soils, bottom and river sediments). The attention was paid to the effect of soil/sediment properties on acetochlor sorption and desorption.

Experimental

Chemicals and Sorbents

Experiments were conducted with analytical grade acetochlor (purchased from Sigma Aldrich Kft.), a relatively non-polar compound ($\log K_{ow} = 2.5$) with water solubility 223 mg dm^{-3}at 20 °C[1].

Eight surface soils (denoted as A1–8) were collected from cultivated sites in Slovakia. Moreover, one soil collected from C horizon at the depth of 2 m (SS), three bottom sediments (BS1–3), and two river sediments (RS1–2) were used. Basic properties of the soils and sediments studied are shown in Table I.

Sorption/Desorption Experiments

Sorption experiments were conducted using a batch equilibration method, with two initial concentrations of acetochlor used (1 and 10 mg dm^{-3}). The acetochlor concentration in supernatant solutions after equilibration was measured using high performance liquid chromatography with ultraviolet detection (HPLC-UV) in the National Water Reference Laboratory for Slovakia (Bratislava).

Desorption experiments followed immediately after the sorption experiments with an initial concentration of 10 mg dm^{-3}.

Results

Sorption coefficients for acetochlor are given in Table II. TOC was found to be the main soil/sediment property correlating with the extent of acetochlor sorption ($r = 0.774$; $P < 0.01$). Strong affinity of acetochlor to soil organic matter was reported also by other researchers[2,3].

Table I
Properties of soils and sediments used in the study

	TOC [%]	C_{HA}/C_{FA}	Clay [%]	pH (H_2O)	$CaCO_3$ [%]
A1	4.59	1.09	11.13	6.93	1.40
A2	1.92	1.12	15.32	7.99	2.00
A3	0.89	0.72	22.08	6.76	0.30
A4	2.49	1.07	9.08	7.86	12.2
A5	1.21	1.16	5.75	6.32	N.D.
A6	1.19	1.53	11.00	7.96	2.74
A7	0.48	0.90	0.60	5.76	0.20
A8	1.57	1.12	37.20	6.11	N.D.
SS	0.60	0.95	4.05	8.20	4.10
RS1	2.46	0.42	2.16	7.17	0.30
RS2	3.46	0.87	1.89	7.22	3.60
BS1	3.05	0.89	6.41	7.56	3.80
BS2	1.64	1.62	7.85	7.46	2.20
BS3	6.39	0.78	2.03	6.45	N.D.

The relationship between K_d and total organic carbon content was improved after excluding the soil A8 from the data set ($r = 0.902$; $P < 0.001$). This could be explained by the significantly higher clay mineral content (especially smectites) of this soil as compared with other sorbents. Previous studies of the sorption of organic pollutants in soils have shown that in soils with the high clay mineral/organic matter ratio, the mineral fraction can play an important role in binding organic compounds such as pesticides[4].

The humic/fulvic acid ratio (C_{HA}/C_{FA}) is one of the characteristics of the soil organic matter quality[5]. The results of this study showed a positive correlation between the K_{oc} and the C_{HA}/C_{FA} ratio ($r = 0.578$; $P < 0.05$). Its signifi-

Table II
Sorption coefficients of acetochlor (Kd and Koc) and percentage of acetochlor desorbed (P_{des})

	Initial concentration $C_0 = 1$ mg dm^{-3}		Initial concentration $C_0 = 10$ mg dm^{-3}		
	K_d [dm^3 kg^{-1}]	K_{oc} [dm^3 kg^{-1}]	K_d [dm^3 kg^{-1}]	K_{oc} [dm^3 kg^{-1}]	P_{des} [%]
A1	7.108	155	5.871	128	12.0
A2	3.248	169	2.537	132	27.9
A3	1.358	152	1.036	116	37.5
A4	3.406	137	2.635	106	19.1
A5	2.109	174	1.551	128	35.6
A6	3.344	281	2.999	252	23.0
A7	1.018	212	0.840	175	34.3
A8	6.265	399	5.145	328	27.7
SS	1.062	177	0.918	153	41.0
RS1	3.463	141	2.579	105	27.0
RS2	4.218	122	3.353	97	22.5
BS1	3.020	99	3.081	101	26.4
BS2	3.854	235	3.378	206	26.2
BS3	6.577	103	5.491	86	16.0

cance increased after excluding the soil A8 from the data set ($r = 0.766$; $P < 0.01$).

The method of step-down multiple regression analysis was used to determine the soil properties significantly contributing to the overall acetochlor sorption. The soil A8 appeared to have specific sorption properties, thus it wasn't included in the analysis. The analysis yielded the following equations:

$$K_d = 1.043(\text{TOC }\%) + 1.725(C_{HA}/C_{FA}) - 0.777 \quad (1)$$
$$R^2 = 0.888;\ n = 12;\ P < 0.001;\ SE = 0.67$$

$$K_d = 0.891(\text{TOC }\%) + 1.723(C_{HA}/C_{FA}) - 1.011 \quad (2)$$
$$R^2 = 0.914;\ n = 12;\ P < 0.001;\ SE = 0.497$$

The equations (1) and (2) were acquired when the results obtained at initial acetochlor concentrations of 1 and 10 mg dm^{-3} were used, respectively. The K_d of acetochlor was found to depend significantly on the total organic carbon content of the soil/sediment. The distribution of humus components expressed as the C_{HA}/C_{FA} ratio is also a significant factor influencing the acetochlor affinity to the sorbents.

Desorption of acetochlor from all soils and sediments was less than the amount initially sorbed (Table II). Desorption extent is significantly ($r = -0.81$; $P < 0.01$) influenced by total organic carbon content, with greater organic carbon contents reducing the desorption. Significant inverse correlations were also found between the humic components (C_{HA} and C_{FA}) and P_{des}, but no other significant correlations between P_{des} and soil/sediment properties were observed.

Conclusions

Organic matter appears to be the main sorbent constituent responsible for acetochlor retention in soils/sediments, with both quantitative and qualitative parameters playing important role in its ability to bind acetochlor. The specific appearance of the clay-rich soil A8 in whole analysis implies that mineral surfaces can also significantly contribute to acetochlor immobilization under favourable conditions.

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P94 ECOTOXICOLOGICAL EVALUATION OF THE SLUDGES FROM WASTE WATER TREATMENT PLANTS

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Introduction

The tendency to improve the water quality in the Czech Republic is linked with building up new waste water treatment plants (WWTP), what results in growing production of sewage sludges. Multi-purpose way for efficient utilization or sludge disposal hasn't existed till now.

Sewage sludges are very rich in nutrients and organic matter. This makes the spreading of this kind of waste on land as a fertilizer or an organic soil improver very suitable. Unfortunately, the sludge tends to concentrate heavy metals and organic compounds present in waste waters. The Sewage Sludge Directive (86/278/EEC) regulates sludge use in such way to prevent harmful effects on soil, vegetation, animals and man. In the Czech Republic almost one third of sewage sludges ends as a waste; this could be hazardeous. In Europe hazardous wastes are classified by 14 criteria including ecotoxicity (H 14). Environmental ecotoxicology deals with the potentially harmful effects of chemicals and wastes on organisms. For this purpose various testing organisms and various type of bioassays are used.

The aim of our study was the ecotoxicological testing of sewage sludges from different waste water treatment plants by selected ecotoxicity tests with respect to their intended use. We used following organisms: crustacea *Daphnia magna* and *Thamnocephalus platyurus* and seeds of terrestrial plant *Sinapis alba.* The values of 24h-LC50 and 48h-LC50 obtained for *Thamnocephalus platyurus* and *Daphnia magna* and 72h-IC50 values gained for *Sinapis alba* are the basic data for the ecotoxicological assessment of the sewage sludges and for their classification following the Czech legislation[1,2].

Experimental

Samples of sewage sludges from high-capacity municipal WWTP, situated in Brno, Modřice and from small WWTP in Veterinary and Pharmaceutical University Brno were evaluated. From WWTP Brno, Modřice following sewage sludges were tested:

- anaerobic stabilized sewage sludges (AS)
- dewatered anaerobic stabilized sewage sludges (DWAS)
- dessicated anaerobic stabilized sewage sludges (DSAS)

From local WWTP in Veterinary and Pharmaceutical University Brno primary sludges were tested (PS).

Samples Preparation

All samples were collected in pure plastic bottle and untill testing stored in the dark at 4 °C for less than four days prior the bioassay experiment. Samples were dried at temperature 105 ± 5 °C and sludge dry residue was determinated gravimetrically. Two types of water leaches were prepared from studied sewage sludges. The first batch of samples was prepared in accordance with Czech legislation; the samples were mixed in ratio 1 : 10 (sludge dry residue: deionised water). The second one was diluted with deionised water to have the same dry matter content as anaerobic stabilized sewage sludges from WWTP Modřice (3.89 %). Sample tubes filled with defined quantity of sample and water were shaken at 5–10 rpm (revolutions per minute) for 24 hour at temperature 15–25 °C. After centrifugation the leaches supernatants were removed and filtered using paper filter (5 μm). To assure suitable surroundings for each testing organism the supernatants were enriched by adding specific amounts of salts following OECD Guidelines[3,4].

Principle of Ecotoxicological Tests

The general principle of ecotoxicological tests is the determination of effective concentration (EC50), eventually lethal concentration (LC50) or inhibition concentration (IC50). These concentrations of tested compound (substance, sewage water or leaches of sewage sludges) cause the mortality of 50 % of testing organisms or 50% inhibition of growth rate in relation to control tests. The organisms are exposed to the test substance for a period of 24, 48, and 72 hours in agreement with requirements of a given test. At least five test concentrations should be used. A stepwise procedure involves three steps: the preliminary test, the confirmatory test and the definitive test. The confirmatory test confirms or disconfirms results of limit test. In preliminary test undiluted sewage sludges leaches are used. If results of preliminary test indicate possible ecotoxicity the definitive test follows. The highest concentration in definitive test should preferably result in 100% immobilization (mortality or inhibition) and the lowest concentration tested should preferably give no observable effect.

Application of Ecotoxicity Tests for Sewage Sludges Evaluation

Testing organisms (Daphnia magna, Thamnocephalus platyurus, Sinapis alba.) were used in preliminary test with raw water sewage leaches (WSL) and in definitive tests with various testing concentrations of WSL; 50, 100, 200, 500, 700 ml dm^{-3}. Under the same conditions tests with control group of the same organisms in standard freshwater but without WSL were conducted.

The Daphtoxkit FTM makes use of the dormant eggs of the crustaceans Daphnia magna, which are protected by chitinous capsule called ephippium. Ephipia can be stored for long time without losing their viability. When the testing organisms are needed it is necessary to put chitinous capsule

into specific environmental conditions and during period of about 3 days testing organisms are ready for use. Daphnids aged less than 24 were exposed to the sewage leaching at a range of concentrations 50, 100, 200, 500, 700 ml dm^{-3} in definitive tests for a period of 24 and 48 hours at 20–22 °C and light intensity of 6,000 lux. Immobilization was recorded at 24 hours and 48 hours and compared with control values. The results were analyzed in order to calculate the EC50 at 24 and 48 h. Determination of the EC50 at 24 h is optional.

The Thamnotoxkit FTM uses the testing organism Thamnocephalus platyurus, aged less than 24 hours. The exposition was 24 h at a range of the same concentrations at 20–22 °C and 4,000 lux. The obtained results were analysed and the values LC50 at 24 h were calculated. Two above mentioned tests are alternative tests, whilst the test on Sinapis alba represents standard phytotoxicity test. This root growth inhibition toxicity test was used as a standard OECD test. The high quality seeds of Sinapis alba were exposed for 78 hours to tested solution, at temperature 20 ± 2 °C in the dark. After 78 hours the lengths of hypocotyls of seeds in tested and in control group were measured and values of IC50 were evaluated. For a validation of test it is necessary to apply the following performance criteria; Daphnotoxkit FTM and Thamnotoxkit FTM – number of dead and immobile organisms should not exceeded 10 % in control. In root growth inhibition toxicity test germinability should be more than 90 %.

Results

On the base of the analysis of obtained ecotoxicological data the values of EC50, LC50 and IC50 were evaluated for various samples of sewage sludges. These values made possible the evaluation of sludges ecotoxicity and also facilitated their categorization from the point of view of the leachability. Table I summarizes values of 24h-EC50 or 24h-LC50 for the most sensitive testing organisms, which form the basis for evaluation and categorization of sewage sludges.

Table I
The ecotoxicity of sewage sludges and their classification

Sewages sludges	24h-EC50 or 24h-LC50 [ml dm^{-3}]	Class of leachability
AS	22.81	III
DWAS	39.57	II
DSAS	139.64	III
PS	38.17	III

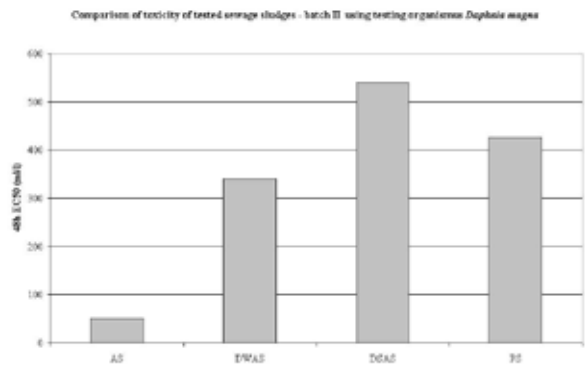

Fig. 1. **Comparison of ecotoxicity of tested sewage sludges using testing organisms *Thamnocephalus platyurus***

Conclusions

Different sewage sludges were tested in respect to their possible hazardous characteristic - ecotoxicity. On the bases of obtained results sewage sludges were classified in term of their leachability. No sample of sewage sludge exhibit ecotoxicity following the Czech legislation.

This work has been supported by grant COST, action 636, project No. OC-183.

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P95 MICROWAVE DESULPHURIZATION OF COAL

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Introduction

Microwave heating has been used in several studies of the coal desulfurization. Desulphurization by microwaves is closely related to the form of sulphur compound in the coal, the physical and chemical structure, as well as the chemical activity of the leachant. The ability of molten NaOH to desulfurize the coal has been known for more than three decades. The process known as molten caustic leaching (MCL) was tested for the first time in the USA. The objective was the removing of the mineral components, pyritic and organic sulfur by the reaction of coal with a mixture of molten sodium and potassium hydroxides. The MCL process was effectively applied for the treatment of the Slovak brown coal[1]. Balaz et al. reported the use of simultaneous grinding and alkaline chemical leaching process (GACL) on brown coal and found that more than 41 % of total sulphur reduction was achieved.

Magnetic methods of mineral removal from coal depend on the difference in the magnetic moment associated with mineral particles and coal. The microwave heating enhances the magnetic susceptibility of the iron mineral, thus rendering it more amenable to the magnetic separation. The effect of microwave heating on magnetic processing of the pyrite was investigated[2], where different size fractions of the pyrite were heated in a microwave oven at 2.45 GHz frequency and different power levels. Ability of coal desulfurization by magnetic separation following microwave heating was also investigated[3]. The microwaves were found as an effective method to selective heating of pyrite in the coal causing the formation of pyrrhotite. It was stated that pyrrhotite could be removed by low-intensity magnetic separation.[4–6]

Experimental

Coal Sample Characterization

In this study, five different coal samples were used. They were ground to less than 3 mm and the representative samples were prepared. The results of analyses of the total sulphur content are listed in Table I.

Microwave and Classical Heating

The microwave heating of investigated coal samples was realized in the microwave oven Whirlpool AVM 434 and Panasonic NN 5251 B with maximum power of 900 W, adjusted for the laboratory purposes for continuos temperature measuring of heated material. The temperature was measured using the contactless thermometer Raynger MX4 in the range 30–900 °C. The classical heating of coal in the mufle oven was realized as well.

Table I
Total sulphur content in the coal samples

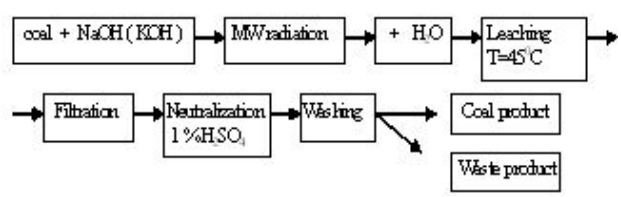
Locality	Grain size [mm]	S_{tot} [%]
Handlová (SR)	–3	1.5
Nováky (SR)	–3	3.0
Cígeľ (SR)	–3	2.27
	1–3	5.35
Sokolov (CR)	0.07–0.5	6.85
	–0.07	7.52
	1–3	0.5
	0.5–1	0.5
Donbas (R)	0.2–0.5	0.49
	0.07–0.2	0.53
	–0.07	0.52

Magnetic Separation

The magnetic separation of the coal samples was carried out by means of a roll-type electromagnetic separator Mechanobr, type 138 T-SEM, intended for dry separation.

Microwave Radiation and Molten Caustic Leaching – RMCL

A mixture of 15 g coal + 15 g NaOH + 10 ml H_2O was used as an input to microwave oven operating in nitrogen atmosphere. Microwave desulphurizastion of coal was realised according to the Scheme 1.



Scheme 1
The RMCL (Radiation and Molten Caustic Leaching) desulphurization of coal

Results

Magnetic Separation of Coal

Coal sample of the grain size 0.05–0.2 mm was heated before magnetic separation in the microwave oven for 10 minutes at the power 900 W and frequency 2.45 GHz. The results of the magnetic separation after microwave radiation are listed in Table II.

The magnetic separation of the coal sample was ineffective for our conditions.

The Molten Caustic Leaching of Coal

Effect of ratio of NaOH and coal

The effect of ratio of NaOH and coal from the Cígeľ locality after the microwave radiation (2.5 minutes) on reduction of the total sulphur content is displayed in Fig. 1.

Table II
The results of coal magnetic separation after the microwave radiation

Grain size [mm]	Magnetic induction [T]	Mass yield of magnetic product [%]	Recovery of S_{pyr} in mag. prod. [%]
	0.13	4.32	39.6
	0.24	4.72	43.2
0.05–0.2	0.42	10.80	44.7
	0.51	11.54	45.5

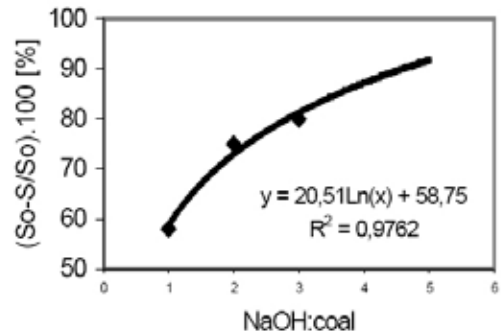


Fig. 1. **The influence of ratio of NaOH and coal on the total sulphur content in coal sample (Cígeľ) after RMCL process, So-content of sulphur basic sample**

From the obtained results, it is possible to state, that 80 % of the total sulphur content is possible to remove by ratio of NaOH : coal = 3 : 1.

Effect of reaction time

The time dependence of microwave heating on decrease of the total sulphur content in coal from the Cígeľ locality by ratio of NaOH : coal = 1 : 1 is displayed in Fig. 2. The sulfur removal increased to 76 % within 5 minutes and reached 85 % at 10 minutes, but after 10 minutes, the change was not significant.

The total sulphur content decreased from 2.27 % to 1.0 % (56 %) in the mufle oven at the temperature 380 °C (within reaction time 40 minutes). The main difference between the thermal and microwave heating was the extremely short time for desulphurization in the case of microwave experiments.

Effect of the coal deposits

The dependence of sulphur content in the coal samples from the localities Cígeľ, Handlová, Nováky in RMCL proces is displayed in Fig. 3. The conditions of the microwave heating were following: temperature 380–400 °C, time 3 minutes, the ratio of NaOH : coal = 1 : 1.

It can be seen, that the effect of microwave pretreatment on reduction of the total sulfur content in samples from Nováky and Cígeľ (52–56 %) is higher than in sample from Handlová (28 %).

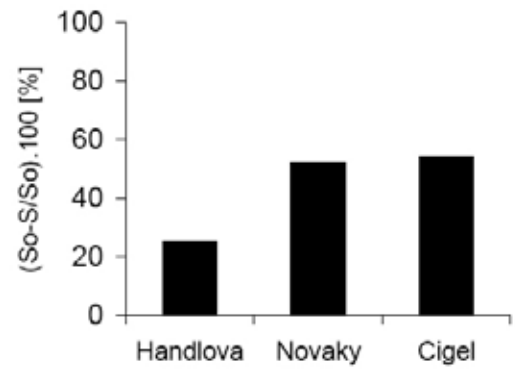


Fig. 3. **RMCL for coal samples from Handlová, Nováky, Cígeľ**

Effect of the particle size

The influence of particle size on chemical desulfurization process was also studied. The representative samples were divided in fractions of 1–3, 0.5–1, 0.07–0.5, < 0.07 mm and microwave heating of samples from Donbas (500 W and 6 minutes) and Sokolov (750 W and 3 minutes) was realized. The results are given in Figs. 4. and 5.

The decrease about 25 % of the total sulphur content was in the sample from Sokolov eminent. The influence of graine size was negligible.

The decrease about 35 % of the total sulphur content in black coal from Donbas locality for graine size 1–3 mm was observed.

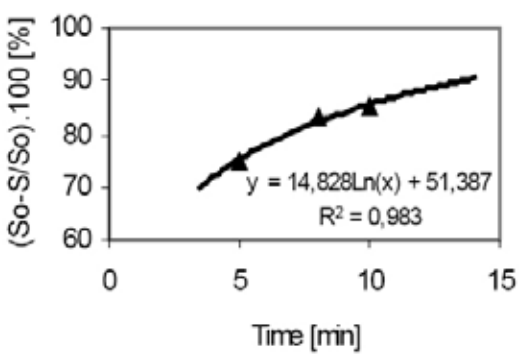


Fig. 2. **The change of total sulphur content of the coal samples with time**

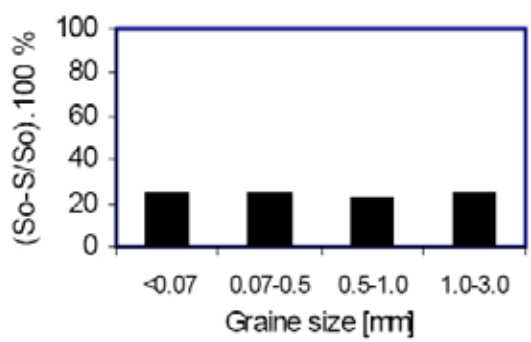


Fig. 4. **The decrease of the total sulphur content in sample from Sokolov versus different size fraction in RMCL process**

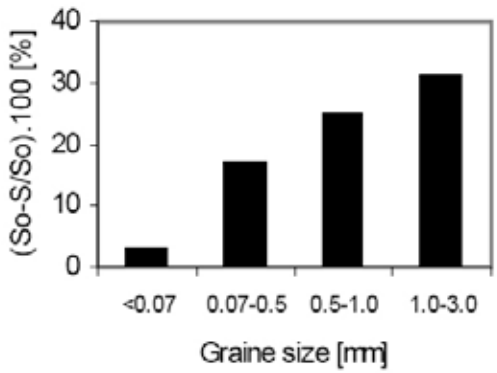


Fig. 5. **The decrease of the total sulphur content in sample from Donbas versus different size fraction in RMCL process**

Conclusions

Microwave heating of the coal is advantageous for the subsequent desulphurization. Conventional procedure of coal desulphurization requires heating period about 40 minutes and microwave procedures reduces this heating period for a few minutes. The use of microwave radiation for the desulphurisation of coal displays potential and may be soon a commercial reality. This would allow the use of high sulphur coals in an environmentally and economically sound way.

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P96 THE BIOLOGICAL ACTIVITY OF 16α(*H*)-PHYLLOCLADANE ISOLATED FROM SLOVAK BROWN COAL

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Introduction

The coal research brings novel opportunities for non-fuel utilization. One of the promising ways is extraction and isolation of organic compounds (diterpenes, humic acids) with potential biological activity. The Slovak coals (Nováky lignite and Handlová brown coal) contain high amounts of tetracyclic diterpene hydrocarbons[1,2], which can be valuable precursors for pharmaceutical products. Kauranes and phyllocladanes are important diastereoisomeric tetracyclic diterpene biomarkers. Tetracyclic diterpene derivates isolated from plants show a strong biological activity and they are active components of many plant medicaments and extracts used in folk medicine. Kauranes exhibit an anti-microbial, anti-HIV, anti-inflamatory, anti-fertility, antiparasitic, insect anti-feedant, antifungal and cytotoxic activities[3]. Phyllocladanes are very rare in the nature; nevertheless, they were also identified and isolated from several plants. Orizaterpenol (phylloklad-15-en-6β,14βdiol) from the rice hull of *Oryza sativa* shows cytotoxicity against murine leukemia cells[4]. The use of phyllocladane diterpenoids for plant growth promotion and/or retardation is already patented[5].

The biological activity of tetracyclic diterpenoids isolated from the geological samples has not been studied yet, therefore the main goal of our work was to isolate tetracyclic diterpane from the coal and test its biological activity *in vitro*.

Experimental

Isolation of 16α(*H*)-Phyllocladane from Slovak Brown Coal

The brown coal (50 kg) was collected in several places of the Handlová mining area (Fig. 1.) and a representative sample was obtained by quartation.

Before the extraction; the Slovak brown coal from Handlová was physically treated on hydrocyclone[7] to obtain quality carbon product with A^d (ash content) = 7.70 % wt., W^a (moisture) = 12.26 % wt., C^d = 61.6 %, H^d = 5.6 %, N^d = 0.7 %, S^d = 1.2 %, O^d = 23.2 %. Further, the coal (20 g)

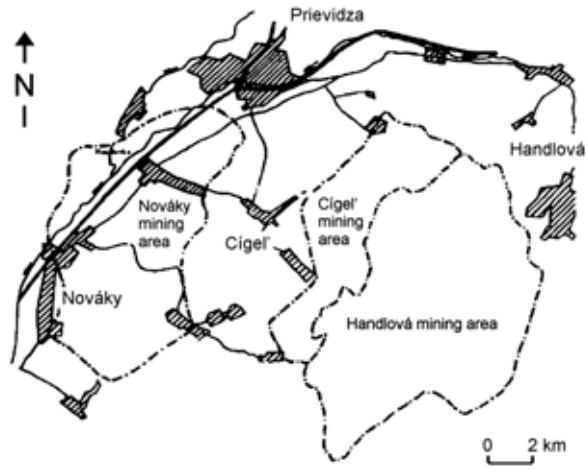

Fig. 1. **The situation map of Handlová-Nováky coal basin with Handlová mining area in central part of Slovakia (adapted from ref.[6])**

was extracted with dichloromethane (250 ml) in microwave oven (Wirlpool AVM 434, 500 W, 2.45 GHz) in distilling flask with reversing reflux system (20 min). After the extraction, the mixture was filtered and the solvent was evaporated. The crude extract (367 mg) was re-extracted on SPE column (Chromabond SiOH, Macharey – Nagel GmbH + Co. KG) with n-hexane (100 ml). The solvent was evaporated and the purified SPE extract (112 mg) was further separated by column chromatography on silica gel (5 g of Kieselgel 60, granularity 0.06 – 0.2 mm, Carl Roth GmbH + Co. KG) with 4 × 20 ml of hexane to four fractions denominated as F1–F4. All fractions were analyzed by TLC and GC/MS. 16α(*H*)-phyllocladane was found in the F1 fraction. TLC analysis of F1 showed presence of a single spot with R_F = 0.91, that contained three compounds: the most abundant component (81.6 %) was 16α(*H*)-phyllocladane; second one was isopimarane (11.2 %) and finally 18-norisopimarane (3.9 %). Unambiguous identification as 16α(*H*)-phyllocladane was achieved by coinjection of the sample and standards in the laboratory of Prof. Bernd Simoneit (Oregon State University, USA). The final isolate (36.7 mg) named as TD1 containing 81.6 % of 16α(*H*)-phyllocladane was used for further biological tests.

Bacterial Strains

All tested $Gram^+$ and $Gram^-$ bacteria (*Enterococcus* spp., β-hemolytic streptococci, *Staphylococcus aureus* CCM 3953, *S. aureus* SA5, *Listeria innocua* LMG 13568 *Enterobacter cloacae* 5139, 19259, 4417, *Klebsiella pneumoniae* 1962, 12506, 5182, 1366, *Serratia marcescens* 19194, *Salmonella enterica* serovar Duesseldorf SA31, *S. enterica* ser. Enteritidis PT4, *Shewanella putrefaciens, E. coli* K88, *E. coli* 6295, 5765) were of human or veterinary origin (isolated from the various wounds, blood, sputum and faeces). The strains were maintained on the appropriate selective media according to ISO.

Spot Test

The antimicrobial activity was determined by the diffusion agar spot test[8]. For the testing, TD1 was dissolved in n-hexane and the various concentration ranges of TD1 (600 mg ml^{-1}; 300 mg ml^{-1}; 150 mg ml^{-1}; 75 mg ml^{-1}) and several volume doses were used; 5 µl is optimal dosage to test on solid agar containing the indicator strain. The solvent n-hexane was used as a control. After 24 hours of incubation at 37 °C, the inhibitory zones were evaluated. MICs (minimal inhibitory concentrations) were expressed as the lowest concentration of TD1 inhibiting the growth of the indicator bacteria (mg ml^{-1}).

Cell Line

The human hepatoma cell line HepG2 was generously provided by Prof. Andrew R. Collins (University of Oslo, Norway). HepG2 cells were maintained in William´s modified medium supplemented with 10% fetal calf serum and antibiotics (penicillin 200 U ml^{-1}, streptomycin and kanamycin 100 µg ml^{-1}) at 37 ºC in humidified 5% CO_2 atmosphere.

Cell Treatment

Hep G2 cells were grown for 48 h until semi-confluency, prior to exposition to TD1 and positive control. TD1 was dissolved in ethanol at 40 ºC freshly before use and added to culture medium to reach the final concentrations ranging from 0.045 to 0.5 mg ml^{-1} for 24 hours. The concentration of the solvent never exceeded 0.1 %. Control cells were exposed to 0.1% ethanol. Cells were exposed to hydrogen peroxide (150 µM), the positive control, on ice in dark for 5 minutes.

Single Cell Gel Electrophoresis (SCGE)

The procedure of Singh et al.[9], modified by Collins et al.[10] and Slameňová et al.[11] was followed. The data were evaluated statistically by the Student's t-test.

Results and Discussion

The details of isolation and structure elucidation of 16α(*H*)-phyllocladane (Fig. 2.) was published previously[2,12].

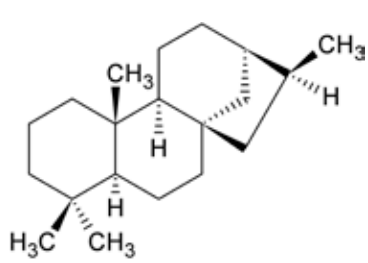

Fig. 2. **The structure of 16α(H)-phyllocladane**

Antimicrobial Activity

TD1 inhibited the growth of 3 Gram$^-$ bacteria (*Kl. pneumoniae* 5182, 1366; *E. coli* 5765) among 19 tested strains. MIC value for these strains is 300 mg ml^{-1}. Others tested bacteria were not sensitive to TD1. The antibacterial activity of tetracyclic diterpene (ent-kauren-19-oic acid) was detected particularly against Gram$^+$ bacteria[13]. In our case, this is the primary and first unique result associated with inhibitory activity of phyllocladane against Gram$^-$, pathogenic bacteria such as *Klebsiella* spp. It is necessary to note that for the further practical usage, the selectivity of the potential pharmaceutical compound is one of the principal conditions.

Genotoxicity

Based on previous cytotoxicity experiments (data not shown), the genotoxic activity of TD1 was evaluated at concentrations 0.045–0.5 mg ml^{-1} (Fig. 3.). In general, only a weak cytotoxicity (~ 20%) was determined at these highest concentrations.

A linear dose dependent increase of DNA migration was detected in TD1-exposed cells ($r^2 = 0.8732$). A mild but statistically significant increase of DNA damage was found at concentrations 0.25–0.5 mg ml^{-1} ($p < 0.05$–0.01). There are limited data concerning the toxicity of phyllocladane-type diterpenes. Recently, Chung et al.[4] reported a strong cytotoxicity of orizaterpenol in P388cells.

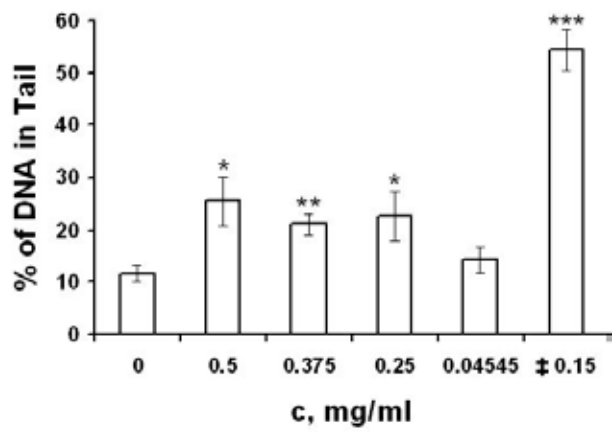

Fig. 3. **The level of DNA damage induced by TD1 in HepG2 cells. Cells were exposed to various concentrations of TD1 for 24 hours. ‡ – positive control – H_2O_2 (0.15 mM). Significantly different from control cells determined by Student's t-test, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$**

Conclusions

Biological activity of TD1 (containing 81.4 % of 16α(*H*)-phyllocladane) from Handlová brown coal was assayed in vitro using pathogenic bacteria and the human hepatoma cell line HepG2. The selective antimicrobial effect against Gram$^-$ strains of *Kl. pneumoniae* 5182, 1366, *E. coli* 5765 was determined with MIC up to 300 mg ml^{-1}. In addition, TD1 induced DNA damage in human tumor cell line HepG2 without strong cytotoxicity. Further search for biological active compounds from Slovak coal and others biological tests will be carried out.

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