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Enhancement of Drought Resistance in Wheat and Corn by Nanoparticles of Natural Mineral Analcite

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Abstract. The effect of the application of nanoparticles of analcite to soil (at 0, 500, 1000 and 1500 mg L^{-1}) on drought resistance of wheat and corn seedlings was studied in pot experiments. The dependence of the analcite effect on soil moisture level and type was also evaluated. For this, three levels of soil moisture were modeled: 20%, 40% and 60% field capacity. The following types of soils were studied: greenhouse soil mixtures, gray podzolic and sandy soils. The positive effect has been established for all doses of analcite tested: seed germination, seedlings growth criteria as well as content of photosynthetic pigments increased, while characteristics of water balance less deviated from the norm under water deficit. Application of analcite caused sharp accumulation of protective antioxidants (flavonoids, carotenoids) and activation of catalase (in corn) under soil drought. While proline content decreased. The dependence of analcite effect on its dose, soil moisture level and type as well as species of tested plants was discussed.

Key words: bioactive silicon, analcite, nanoparticles, drought resistance, seedlings, *Triticum aestivum, Zea mays*

Introduction

Recent studies have shown an important role of silicon in the plant life. Silicon is widely present in plants in amounts equivalent to or higher than phosphorus and magnesium (NISHIMURA *et al*., 1989). This element is fairly common in nature. However, continued exploitation of agricultural lands, along with the use of mineral fertilizers led to leaching of natural silicates from the soil. Today concentration of bioactive silicon in the soil is recognized to be a factor limiting the performance of crops especially such as rice and sugar cane (MA *et al*., 2006).

The positive effect of bioactive silicon on plants is widely regarded in the scientific literature (EPSTEIN, 2001). Exogenous application of bioactive silicon was shown to promote growth and productivity of crops and to improve the quality of agricultural products. This is due primarily to improved mechanical properties of plant tissues, enhancement of systemic resistance to abiotic and biotic stress factors and the impact of Si on the uptake of N, P, K and Mo by plants (KOZLOV, 2007).

Analcite $[AlSi_2O_6]$-H_2O is a natural mineral of volcanic tuffs. It was shown to have marked positive effect on the functional state of living organisms. The inclusion of analcite to fertilizer promote root development, improves soil agro-physical characteristics by increasing

moisture level and creating chemical depot for macro- and micronutrients (ZAIMENKO, 2008).

Despite many recent reports describing benefits of application of silicon containing minerals in ecological agriculture, the physiological mechanisms of their effects on crops remained undefined and controversial up till now. This prevents wide implementation of silicon in agriculture.

Therefore, the present investigation was undertaken to study the impact of analcite on drought resistance in wheat and corn seedlings depending on the dose of the mineral, soil type and moisture level.

Wheat is considered the first strategic food crop in Ukraine. It is the basic staple food for bread making. Corn is also one of the key grain crops with growing popularity in this region. In Ukraine wheat and corn crops are sometimes exposed to drought at various stages of growth. However the most vulnerable to drought these crops are at seedling stage.

Material and Methods

Seed materials. Wheat (*Triticum aestivum* L., cv. "Pereyaslavka") and corn (*Zea mays* L., hybrid "Titan"), which represented C_3 and C_4 types of fixation of carbon during photosynthesis, respectively, were used in this study.

Analcite application. Analcite nanoparticles (with size of 100 micron) were added to soil at 0, 500, 1000 and 1500 mg L^{-1} just before sowing seeds**.**

Soil types and moisture levels. Three types of soil were used: (**1**) greenhouse soil mixture consisting of compost, peat, leaf soil and sand (1:1:1:1), (**2**) gray podzol and (**3**) sand + Hellriegel nutrient solution. Soil were sieved through a 2 mm sieve and sterilized in an oven at 100 ^{o}C. Moisture level was maintained by gravimetric method at 20%, 40% and 60% field capacity (FC). Corn and wheat were cultivated for 21 and 28 days, respectively, at 22-30 °C temperature, natural sunlight level and 60-75% relative humidity.

Measurements. Success of adaptation of corn and wheat seedlings to water deficit was assessed by characteristics of growth and development (% of germinated seeds, dry weight of shoots and roots), water balance (relative water content and water deficit in the foliar tissues, transpiration rate), content of photosynthetic pigments, as well as content of protective biomolecules (proline, flavonoids) and catalase activity. Percentage of germinated seeds was counted on 3-5 days after sowing. The transpiration rate was determined by registering changes in weight of cut transpirating leaves for short time intervals (TRETYAKOV, 1990). Relative water content and water deficit were measured by gravimetric method (GRYGORYUK *et al.*, 2003). Proline was extracted from freshly cut leaves by 3% sulfosalicylic acid. Its quantitative content was determined using qualitative reaction with ninhydrin on spectrophotometer "Spekol 11" (Carlzeiss / Jena, Germany) (STATSENKO & BUTYLKIN, 1999). Photosynthetic pigments were extracted from leaves by dimethylsulfoxide. Their quantitative content was determined using spectrophotometer "Spekol 11" (HISCOX & ISRAELSTAM, 1979). Catalase activity was determined by the method of PLESHKOV (1985). Flavonoids were extracted with 70% ethanol, their quantitative content was determined spectrophotometrically using qualitative reaction with $AlCl_3$ (KOMAROVA *et al.*, 1998). The results presented in the tables are the means of four replications. The data were statistically analyzed using the least significant difference (LSD) test ($p<0.05$). The effect of analcite dose, soil type, moisture level, and species of tested plants on their adaption to soil drought was assessed using analysis of variance (ANOVA) and correlation analysis with the help of Statistica 6.0 software.

Results and Discussion

Seed germination

Under moderate and especially low soil moisture seed germination of wheat and corn noticeably decreased. Supplying of analcite nanoparticles to substrate promoted seed germination of corn and wheat (Table 1). The effect size, as a rule, positively correlated (correlation coefficient $R=0.21$, $p<0.05$) with

the dose of the mineral and inversely with soil moisture level (R=-0.49, p<0.05). The stimulation observed on wheat seeds was more pronounced as compared to corn seeds under low soil moisture conditions. At 60% FC the opposite tendency was observed. No signifycant correlation between the degree of analcite protective effect and soil type was observed.

Growth parameters

In treatments with limited soil moisture, the growth of shoots of wheat and corn seedlings was markedly suppressed as compared to seedlings grown on substrate moistened to 60% FC (Table 2). The development of root system showed the opposite tendency for wheat grown on soils one and two and corn grown on soil 1. On the sandy substrate moistened to 20% FC, the growth of the root system of wheat and corn seedlings was also significantly depressed. The shoot/root ratio was generally lower in seedlings grown under limited soil moisture. This is a typical morphological adaptation of plants to water deficit in the soil.

Supplying of analcite nanoparticles to the soil increased phytomass accumulation by wheat and corn seedlings. For wheat, the increments in dry weight of shoots often were highly significant in comparison with plants grown without analcite. While the promoting effect of analcite on root phytomass was significant only in wheat seedlings grown on sandy substrate. For corn, both shoot and root dry weights were highly enhanced by analcite application in all treatments. Plant growth is an integral indicator of their vitality. Therefore, observed improvement of shoots and roots phytomass accumulation evidenced the restoration of basic life functions in wheat and corn seedlings under soil drought and the further amelioration of seedlings vigor under optimal soil moisture conditions (60% FC). The size of analcite effect on phytomass criteria positively correlated with its dose (R=0.29, p<0.05), soil fertility (R=0.12, p<0.05) and inversely – with soil moisture level (R=-0.67, p<0.05). The growth of corn seedlings was more pronouncedly stimulated by analcite application as compared to wheat.

Table 1. Wheat and corn seed germination (%) depending on analcite dose, soil type and moisture level. LSD – least significant difference at P<0.05

Plant species		Wheat			Corn		
Soil moisture, % FC	Analcite dose, mg L^{-1}	Soil 1	Soil 2	Sand	Soil 1	Soil 2	Sand
20	0	30.0	75.0	67.5	57.5	42.2	55.4
20	500	62.5	92.5	80.0	65.0	55.6	69.8
20	1000	80.0	97.5	90.0	65.0	57.8	74.0
20	1500	87.5	95.0	92.5	74.0	64.5	75.0
LSD		*1.63*	*1.22*	*1.87*	*0.99*	*0.71*	*0.70*
40	0	75.0	85.0	87.5	67.5	58.4	63.5
40	500	77.5	95.0	90.0	75.0	72.1	65.0
40	1000	82.5	92.5	97.5	80.0	79.5	72.7
40	1500	85.0	97.5	96.7	85.0	84.3	80.0
LSD		*2.42*	*1.51*	*1.66*	*0.97*	*0.66*	*0.98*
60	0	82.5	92.5	92.0	77.5	72.3	75.5
60	500	85.0	93.5	95.0	79.0	76.5	80.0
60	1000	82.5	95.0	98.0	87.5	85.0	86.8
60	1500	88.0	93.5	97.5	92.5	88.4	90.0
LSD		*2.41*	*1.02*	*1.57*	*1.11*	*0.57*	*0.52*

Table 2. Mean dry weight per plant of wheat and corn seedlings (mg) depending on analcite dose, soil type and moisture level. LSD – least significant difference at P<0.05

Soil moisture, % FC	Analcite dose, mg L^{-1}	Soil 1			Soil 2			Sand		
		Roots	Shoots	Shoot/ root	Roots	Shoots	Shoot/ root	Roots	Shoots	Shoot/ Root
Wheat										
20	0	5.4	3.2	0.6	5.6	2.3	0.4	10.6	10.0	1.1
20	500	5.3	4.3	0.8	5.6	2.6	0.5	25.5	13.6	1.9
20	1000	5.5	7.8	1.4	5.8	2.9	0.5	24.7	12.9	1.9
20	1500	5.6	7.1	1.3	5.5	6.0	1.1	27.7	13.8	2.0
LSD		*0.5*	*0.5*	*0.4*	*0.4*	*0.6*	*0.4*	*0.8*	*0.7*	*0.5*
40	0	4.0	10.4	2.6	3.6	13.8	3.8	17.7	15.6	1.1
40	500	4.0	10.9	2.8	3.5	14.5	4.2	29.0	16.5	1.8
40	1000	3.8	13.5	3.6	3.4	17.4	5.1	28.7	16.3	1.8
40	1500	3.9	12.8	3.3	3.3	17.4	5.3	29.6	18.0	1.6
LSD		*0.4*	*0.7*	*0.4*	*0.5*	*0.6*	*0.5*	*0.4*	*0.5*	*0.3*
60	0	3.7	20.3	5.5	3.4	17.9	5.2	19.0	18.3	1.0
60	500	3.6	20.0	5.6	3.9	25.2	6.5	28.6	19.2	1.5
60	1000	3.7	20.8	5.6	3.9	25.3	6.4	33.7	18.6	1.8
60	1500	4.0	21.1	5.3	4.0	24.7	6.2	32.8	19.2	1.7
LSD		*0.7*	*0.4*	*0.3*	*0.2*	*0.6*	*0.2*	*0.9*	*0.5*	*0.5*
Corn										
20	0	65.5	23.6	0.4	44.2	13.3	0.3	26.7	13.3	0.5
20	500	70.8	38.0	0.5	55.6	27,2	0.5	41.4	28.6	0.7
20	1000	90.9	36.6	0.4	54.8	29.0	0.5	39.4	32.5	0.8
20	1500	83.3	38.5	0.5	55.5	27.5	0.5	33.1	38.8	1.2
LSD		*1.3*	*0.8*	*0.8*	*0.9*	*1.5*	*1.0*	*0.7*	*0.5*	*0.4*
40	0	47.4	21.1	0.4	38.6	21.4	0.6	63.3	32.2	0.5
40	500	49.4	29.4	0.6	55.5	35.5	0.6	109.3	49.3	0.5
40	1000	49.4	32.7	0.7	52.5	35.8	0.7	83.5	48.0	0.6
40	1500	50.9	39.5	0.8	68.6	33.9	0.5	80.5	49.5	0.6
LSD		*0.3*	*0.6*	*0.3*	*0.4*	*1.2*	*0.5*	*0.9*	*1.0*	*0.8*
60	0	42.4	35.2	0.8	89.0	44.0	0.5	60.6	41.9	0.5
60	500	45.4	40.0	0.9	93.9	48.3	0.5	96.3	43.7	0.5
60	1000	51.4	40.5	0.8	104.5	92.7	0.9	90.5	55.8	0.6
60	1500	52.9	46.2	0.9	96.3	89.6	0.9	107.5	70.0	0.7
LSD		*0.7*	*0.8*	*0.6*	*0.5*	*2.2*	*0.6*	*0.5*	*0.5*	*0.5*

Water balance

Under water deficit significant dehydration of foliar tissues was observed in seedlings of wheat and corn (Table 3). Application of analcite led to normalization of water metabolism under water deficit. At 60% FC some reduction of water deficit and increase in transpiration rate (statistically not significant) in leaves of plants treated with analcite were observed. This evidenced amelioration of water balance and gas exchange processes in wheat and corn at all levels of soil moisture by analcite application. The effect size positively correlated with the dose of the mineral and soil fertility (R=0.16 and R=0.12, respectively, p<0.05) and inversely – with soil moisture level (R=-0.26, p<0.05).

Table 3. Characteristics of water balance in leaves of wheat and corn seedlings depending on analcite dose, soil type and moisture level. LSD – least significant difference at P<0.05. WC – relative water content (%), WD – water deficit (%), TR – transpiration rate (mg/cm²·h).

Soil moisture% FC	Analcite dose, mg L^{-1}	Soil 1			Soil 2			Sand		
		WC	WD	TR	WC	WD	TR	WC	WD	TR
Wheat										
20	0	58.2	74.0	1.22	70.7	64.0	0.42	72.2	31.0	0.90
20	500	78.5	43.1	1.82	82.6	57.2	0.63	88.6	21.7	1.34
20	1000	79.2	30.7	2.71	84.7	44.0	0.80	88.8	10.1	1.33
20	1500	81.6	37.5	3.10	87.0	40.4	1.22	89.2	6.5	1.46
LSD		*5.3*	*4.2*	*0.41*	*3.7*	*2.4*	*0.19*	*1.8*	*2.9*	*0.29*
40	0	80.7	14.8	1.60	87.2	13.4	2.39	85.0	15.1	0.96
40	500	91.2	10.7	1.96	91.5	6.5	2.45	91.8	10.6	1.87
40	1000	90.0	9.0	2.54	92.1	4.0	3.20	91.7	10.7	2.11
40	1500	91.6	8.1	3.37	92.5	4.4	3.78	93.1	7.0	2.12
LSD		*1.8*	*2.8*	*0.37*	*2.0*	*2.4*	*0.35*	*2.7*	*1.4*	*0.13*
60	0	90.9	7.0	2.39	93.6	11.3	3.83	90.8	3.9	3.12
60	500	91.5	5.1	3.00	92.2	5.0	4.04	92.7	1.8	3.30
60	1000	91.2	4.5	3.67	92.6	3.8	4.68	92.4	1.9	3.31
60	1500	91.7	2.2	3.85	93.6	2.2	4.63	92.5	1.5	3.25
LSD		*2.1*	*2.4*	*0.66*	*4.1*	*3.9*	*0.85*	*1.4*	*2.0*	*0.14*
Corn										
20	0	77.4	61.1	0.48	58.5	80.7	0.85	74.9	49.0	0.67
20	500	82.4	41.0	0.66	76.7	67.5	1.02	88.9	28.0	0.88
20	1000	84.6	39.4	0.61	79.6	71.9	1.14	90.0	19.9	0.98
20	1500	85.2	31.4	0.69	77.1	65.3	1.17	90.2	13.6	1.05
LSD		*1.7*	*2.3*	*0.23*	*2.9*	*3.3*	*0.34*	*1.9*	*3.8*	*0.26*
40	0	85.2	29.8	0.81	87.1	17.8	1.10	87.3	30.8	0.97
40	500	89.6	25.2	0.89	91.7	9.7	1.34	91.3	19.6	1.12
40	1000	90.8	19.2	1.09	92.7	5.7	1.41	91.6	16.6	1.24
40	1500	91.0	17.8	1.09	93.5	3.8	1.55	92.5	10..8	1.22
LSD		*3.9*	*1.6*	*0.21*	*2.6*	*5.2*	*0.33*	*1.9*	*2..4*	*0.24*
60	0	91.2	13.8	1.78	91.9	16.6	2.35	89.6	18.0	1.97
60	500	92.1	4.1	1.71	92.0	10.6	2.45	92.3	14.0	2.02
60	1000	92.3	3.8	1.91	92.7	6.0	2.59	92.5	8.2	2.13
60	1500	92.7	2.5	1.91	92.4	6.1	2.88	93.4	7.0	2.14
LSD		*2.9*	*1.9*	*0.4*	*1.4*	*0.1*	*0.48*	*3.1*	*2.5*	*0.27*

Photosynthetic pigments

Photosynthesis is the main metabolic process that provides biomass accumulation in plants. Its rate depends on the content of photosynthetic pigments in foliar tissues, their composition and ratio. The results of the analysis of the content of chlorophylls a and b as well as carotenoids are shown in Table 4. Under soil drought a significant decrease in the content of photosynthetic pigments (chlorophylls a and b, carotenoids) and the chlorophyll a to chlorophyll b ratio

in leaves of wheat and corn seedlings was observed.

Analcite application led to a sharp increase in content of photosynthetic pigments (chlorophylls a, b and carotenoids) and chlorophyll a/b ratio. No statistically significant correlation between the size of analcite effect with its dose and soil fertility was found. While inverse correlations between the size of analcite effect and soil moisture ($R=-0.31$, $p<0.05$) was established.

Thus, the application of analcite nanoparticles to substrate promoted induction of synthesis of photosynthetic pigments in leaf tissues of wheat and corn or modified ways of biosynthesis of photosynthetic pigments in the direction of enhancement of drought resistance of plants. Increase of chlorophyll a/b ratio in photosystems indicated the reducing of stress. An important role of carotenoids in the biosynthesis of ABA and maintaining the viability of plants under stressful conditions has been described in the scientific literature (MALUF *et al*., 1997). It is also known that carotenoids are components of the antioxidant systems involved in protecting membranes from damaging effects of free radicals produced during water stress (PINZINO *et al*., 1999). Since photosynthetic pigments are highly vulnerable to the oxidative stress, a significant increase in carotenoid content in case of analcite application to substrate is very important to protect the photosynthetic system and maintaining its operation under soil drought.

Proline content

Accumulation of proline in plant cells facilitates storage of water and is an important physiological mechanism of plant adaptation to drought (KUZNETSOV & SHEVYAKOVA, 1999). In treatments with limited soil moisture the proline content noticeably increased (Table 5).

Analcite application to the soil led to reduction of proline content in leaves of wheat and corn seedlings in all treatments. This indicated a lower stress level in seedlings treated with analcite and pointed to the fact that accumulation of proline is not related to the manifestation of the protective action of analcite. There was not found any statistically significant correlation between the size of analcite effect with its dose and soil type and moisture level.

Catalase activity

Activation of catalase in response to stress is a key process in the development of defense reactions in plant cells. Catalase breaks down hydrogen peroxide to oxygen and water, thus preventing its toxic effect to plant organism (WILLEKENS, 1997).

Application of analcite nanoparticles to the substrate resulted in the opposite effect on catalase activity in leaves of wheat and corn grown under soil drought. In the first case, the activity of catalase decreased proportionally to analcite dose, in the second - grew. At 60% FC application of analcite to a substrate led to reduction of catalase activity in corn leaves while in wheat it did not changed. This evidenced the greater sensitivity of corn catalase to analcite application as compared to wheat. No statistically significant correlation between the size of the analcite effect with its dose, soil fertility and moisture level was revealed.

The content of flavonoids

The content of flavonoids in the leaves of corn and wheat seedlings parabolically depended on the level of soil moisture, with a maximum at 40% FC. This was the evidence of the involvement of flavonoids in the responses of the tested plants to water deficit in the soil. The lowest level of flavonoids in leaf tissues of wheat and corn was observed at 20% FC. (Table 7). Under such soil moisture conditions analcite application stimulated accumulation of flavonoids in the leaves of wheat and corn seedlings. This pointed to activation of the corresponding defensive antioxidant system in response to drought stress by analcite nanoparticles. In treatments moistened to 40% and 60% FC analcite application led to the reduction of flavonoid content in leaves. This reaction pointed to the lower stress level in plants treated with analcite.

Table 4. The content of photosynthetic pigments (mg·g^{-1}) dry weight in leaves of wheat and corn depending on analcite dose, soil type and moisture level. LSD-least significant difference at P<0.05

Soil moisture %FC	Analcite dose, mg L^{-1}	Soil 1			Soil 2			Sand		
		Chl a	Chl b	Car	Chl a	Chl b	Car	Chl a	Chl b	Car
Wheat										
20	0	2.7	1.6	1.3	5.5	4.1	1.0	5.8	3.2	3.0
20	500	6.3	2.7	2.4	9.0	5.3	1.8	7.7	3.9	4.0
20	1000	6.9	2.7	2.4	10.3	4.7	3.1	8.6	3.6	3.7
20	1500	13.0	5.2	5.9	10.5	3.9	3.3	8.4	3.8	3.9
LSD		*1.3*	*0.5*	*0.3*	*0.4*	*0.5*	*0.2*	*1.4*	*0.4*	*0.4*
40	0	16.3	10.8	7.6	11.4	5.8	3.8	12.3	6.7	4.2
40	500	18.4	10.4	7.4	12.1	6.0	4.6	15.7	5.9	5.6
40	1000	17.5	8.3	7.5	14.3	6.5	5.2	13.6	5.5	5.9
40	1500	17.3	8.3	8.2	15.4	6.2	5.0	15.2	5.5	5.9
LSD		*0.8*	*0.7*	*0.8*	*1.1*	*0.5*	*0.1*	*1.1*	*0.3*	*0.6*
60	0	17.0	7.2	8.2	15.0	5.2	4.0	11.6	5.5	4.8
60	500	20.9	10.5	9.7	14.8	5.1	4.0	13.3	4.8	4.5
60	1000	19.9	10.3	8.1	16.6	5.5	4.8	12.9	4.2	4.5
60	1500	19.1	7.6	8.8	16.5	5.8	4.5	13.5	4.8	5.3
LSD		*1.2*	*0.7*	*0.3*	*0.5*	*0.5*	*0.8*	*1.2*	*0.6*	*0.3*
Corn										
20	0	4.5	3.4	2.0	2.6	1.9	1.2	4.2	2.9	1.9
20	500	6.5	5.6	3.2	7.5	4.5	1.8	7.0	3.6	2.1
20	1000	6.0	4.9	4.1	7.2	4.6	2.7	8.9	4.0	3.0
20	1500	6.4	4.6	4.8	7.8	4.9	2.5	9.0	3.8	2.8
LSD		*0.7*	*0.1*	*0.3*	*1.2*	*1.2*	*0.2*	*1.2*	*1.2*	*0.4*
40	0	12.7	6.2	5.6	9.1	4.6	2.1	9.0	5.5	3.0
40	500	17.1	6.0	3.7	10.4	4.4	2.1	12.9	6.1	3.4
40	1000	16.9	5.4	3.8	11.0	3.6	2.6	10.9	4.7	4.3
40	1500	18.0	6.0	3.9	11.7	3.7	3.0	11.6	4.6	4.1
LSD		*1.2*	*0.3*	*0.1*	*1.1*	*1.1*	*0.3*	*2.1*	*2.3*	*0.7*
60	0	11.2	6.2	4.2	13.6	5.1	2.5	11.8	4.3	3.3
60	500	12.8	8.0	3.8	15.0	5.0	2.8	14.1	4.5	3.4
60	1000	13.6	8.7	3.6	16.8	5.7	3.2	11.6	4.3	3.9
60	1500	13.5	7.2	4.2	19.5	6.6	2.9	19.9	5.1	5.0
LSD		*0.5*	*0.2*	0.5	*1.1*	*1.3*	*0.2*	*3.1*	*1.3*	*0.7*

Table 5. Proline content (mg per g of dry weight) in the shoots of wheat and corn depending on analcite dose, soil type and moisture level. LSD-least significant difference at P<0.05

Plant species		Wheat			Corn		
Soil moisture % FC	Analcite dose, mg L^{-1}	Soil 1	Soil 2	Sand	Soil 1	Soil 2	Sand
20	0	8.1	11.2	4.3	3.2	4.5	2.5
20	500	7.3	10.4	4.1	3.3	3.8	1.8
20	1000	4.1	8.1	3.0	2.3	2.5	1.7
20	1500	3.8	6.3	2.3	2.0	2.4	1.7
LSD		*0.4*	*0.5*	*0.3*	*0.3*	*0.4*	*0.4*
40	0	2.6	6.2	2.7	2.1	3.0	1.5
40	500	1.5	5.7	2.8	2.5	2.1	1.3
40	1000	0.9	5.1	2.3	1.5	2.0	1.5
40	1500	1.2	4.4	2.6	0.4	2.3	0.9
LSD		*0.5*	*0.6*	*0.3*	*0.6*	*0.6*	*0.5*
60	0	2.8	3.4	3.2	1.4	2.9	1.9
60	500	2.2	2.8	3.1	1.0	1.1	1.4
60	1000	1.0	2.4	3.0	0.6	1.6	1.6
60	1500	1.4	1.7	3.0	0.9	1.3	1.2
LSD		*0.4*	*0.4*	*0.4*	*0.4*	*0.5*	*0.5*

Table 6. Catalase activity (mmol H_2O_2/min per 1 g of dry weight) in the leaves of wheat and corn depending on analcite dose, soil type and moisture level. LSD-least significant difference at P<0.05

Plant species		Wheat			Corn		
Soil moisture % FC	Analcite dose, mg L^{-1}	Soil 1	Soil 2	Sand	Soil 1	Soil 2	Sand
20	0	36.46	30.95	8.75	6.15	4.43	4.48
20	500	35.42	14.70	6.17	6.68	3.30	4.41
20	1000	24.28	13.90	5.97	7.89	5.65	4.75
20	1500	22.31	12.01	4.38	7.89	8.47	7.23
LSD		*2.4*	*2.7*	*3.1*	*1.8*	2.9	2.4
40	0	20.71	18.75	7.71	10.67	8.44	3.54
40	500	20.71	16.78	6.99	8.87	4.81	3.06
40	1000	20.71	16.78	5.42	14.21	10.84	3.63
40	1500	20.71	12.17	4.50	19.54	10.86	4.21
LSD		*3.1*	*2.7*	*1.6*	*2.2*	*1.9*	*3.2*
60	0	10.83	7.08	5.66	8.98	7.22	3.03
60	500	11.91	6.69	5.66	9.66	9.02	2.68
60	1000	11.91	6.53	5.66	9.66	9.00	1.61
60	1500	11.91	6.04	5.66	7.70	6.19	1.52
LSD		*2.0*	*3.0*	*2.4*	*1.6*	*1.8*	*1.7*

Table 7. The content of flavonoids (mg per 1 g dry weight) in the leaves of wheat and corn depending on analcite dose, soil type and moisture level. LSD-least significant difference at P<0.05

Plant species		Wheat			Corn		
Soil moisture, % FC	Analcite dose, mg L^{-1}	Soil 1	Soil 2	Sand	Soil 1	Soil 2	Sand
20	0	1.46	0.52	3.23	1.55	1.67	1.43
20	500	3.54	1.91	4.35	1.65	7.62	1.65
20	1000	3.97	1.72	6.91	4.49	5.83	1.85
20	1500	6.71	3.29	5.14	4.57	7.16	3.11
LSD		*0.9*	*1.1*	*1.3*	*1.1*	*1.0*	*1.1*
40	0	7.33	3.71	5.92	5.90	9.29	2.48
40	500	7.10	2.41	4.02	2.43	4.75	2.19
40	1000	5.99	1.86	3.99	5.92	5.20	1.98
40	1500	5.94	1.81	3.50	5.26	3.92	1.62
LSD		*1.1*	*1.1*	*0.8*	*1.1*	*1.2*	*1.2*
60	0	6.74	1.26	6.86	3.05	5.83	2.01
60	500	5.07	1.16	4.97	3.44	5.76	1.91
60	1000	5.67	1.68	4.10	3.02	5.08	1.28
60	1500	4.84	1.13	4.54	2.55	5.04	1.26
LSD		*0.8*	*0.8*	*1.0*	*1.1*	*1.1*	*1.0*

No statistically significant correlation between the size of analcite effect with its dose and soil fertility was revealed. However the size of analcite effect inversely correlated ($R=-0.26$; $p<0.05$) with the soil moisture level.

Conclusions

The data presented indicate that the application of nanoparticles of analcite to substrate contributes to the increase in the resistance of corn and wheat plants to drought stress: seed germination, seedlings growth criteria as well as content of photosynthetic pigments increased, while characteristics of water balance less deviated from the norm under water deficit. Analcite application induced a sharp accumulation of protective antioxidants (flavonoids, carotenoids) and activation of catalase (in corn) under soil drought, while accumulation of proline was not related to the manifestation of the protective action of analcite. Analcite is also known to improve water regime of the soil by alteration of agrophysical soil characteristics (ZAIMENKO, 2008).

The effect size of analcite, as a rule, positively correlated with its dose, soil fertility and inversely – with soil moisture level. It also varied depending on species of plants tested. For seed germination and water balance it was greater for wheat, while characteristics of content of photosynthetic pigments, biomass accumulation, catalase activity were more sensitive to analcite application in corn. This was the evidence of the differences in the development of protective responses to drought in wheat and corn.

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Contribution to the Study of the Structure of the Main Ligneous Species in the Preservation of Forest Spaces in the Monts of Saida and Dhaya (Algerian West)

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Abstract. In arid Mediterranean region, the structure of forest stands is the image of their capabilities durability and resistance demonstrated by the rate of land by persistent vegetation recovery rate by stratum and living space, which are useful for understanding the dynamics of vegetation. These three criteria used to classify the different layers according to their importance in bioclimatic zones, the semi-arid and sub-humid, and the most dominant in Algeria. The arborescent and shrub Stratum plays an important role in the two bioclimatic zones points of view recovery; points of view land soil occupation in semi-arid is the under shrub stratum in which premium and shrub Stratum for the sub-humid. Eight principal forest species that dominate the area, their living space depends on density and regeneration capacity, it varies between 28 and 222 square meter depending on the species inducing bad conduct densities and silvicultural work. For that, to preserve these strata with the species composition and ensure stability, a silvicultural work or plantations are necessary.

Keywords: Structure, recovery rates, living space, forest vegetation, sustainability, Monts of Saida and Dhaya (western Algeria).

Introduction

Being threatened by fire, herds and drought, the sustainability of forest formation in Algeria remains a challenging concern. In addition to such degrading factors, there are also the unfavourable physic-chemical characteristics of soil (which consists in a low water holding capacity, a poor organic matter as well as a structural instability). The survival of these forest formations is conditioned by the rate of covering, the land use and the living spaces that are necessary for key species. These latter three elements are significant indicators that call for an appreciation of the features that reveal the importance of both ecological and economic forest formations (BOUDON, 1968).

The covering rate resulting in the species' occupation of living space determines the habitat so vital for each individual timber plant living. In addition, a reference parameter assesses both the vitality and the resilience of species to environmental conditions. These data are important for the Mediterranean region because they directly depend on constraints and anthropogenic climate change. Moreover, it is possible to identify plant species that may play a role in the sustainability of forest formations.

In Algeria, the concentration of more than 80% of forest cover in the semi-arid bioclimatic justifies the study of the species average distribution in living space besides strengthening the role of density in ensuring the survival of these plants. In this regard, BENABDELI (1983) notes that, "Recovery rate and actual occupation of land in forest formations help identify the best planting densities and stands education".

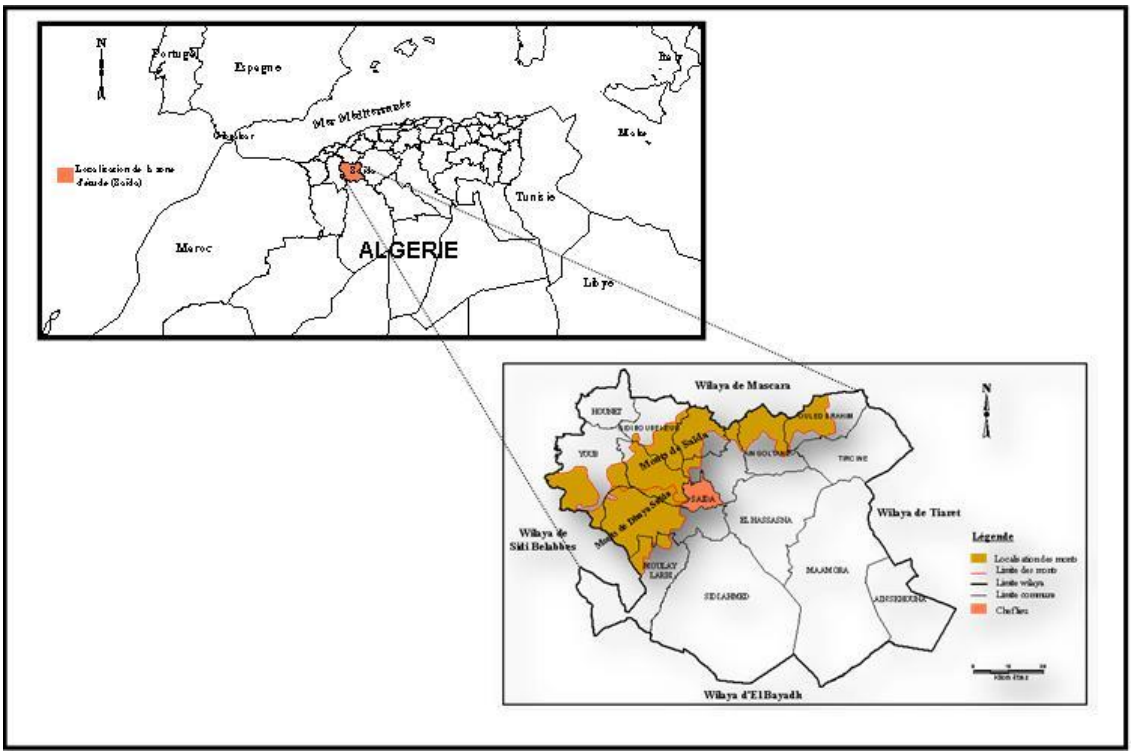

Fig. 1. Delimitation of the Study Area (the Mounts of Saida and Dhaya)

Material and Methods

In each bioclimatic stage and according to the plant grouping type being identified, ten plots of 10 square meter each were delineated and were used for assessing the rate of land by woody plant species contributing to the sustainability of forestry groups. The parameters evaluated were the occupation of space by persistent plants, the recovery per stratum and the living space reserved for each species. This would allow for a better understanding of the behaviour and dynamics of the major forest formations.

Evaluating these three parameters is crucial to assess the forest formations' potential for resistance to various attacks such as by the anthropogenic climate change. This evaluation was done to calculate the land occupation considering all ground species. The space occupation is found by substracting the remaining part of the bare ground. Evaluating the living space is achieved by dividing the area according to the number of dominant species that represent the backbone of the vegetal formation.

The plant communities where the three parameters have been described are for the bioclimatic semi-arid stage for a forest of Aleppo pine and cedar and a forest of Aleppo pine and oak. For the sub-humid bioclimatic stage, there are copses of oak, juniper, and oak coppice with standards of Aleppo pine and holm oak. These are the most dominant forest formations in the forest area of the Dhaya Mountains and Saida Mountains.

Results

The main results obtained concerning the occupancy of space, the rate of land by strata as well as the living space reserved for the main species inform of some techniques

to apply including restocking, replanting and cutting.

Space Occupancy Rate

This is a critical parameter in determining the aspect, the increase, the structure and dynamics of forest formations. It results in an assessment of the recovery rate of each species and each stratum by the projection of the total biomass of the species on the ground. It will confirm the impact of other parameters on the identification and determination of physiognomy (DUVIGNEAUD, 1980).

The rates occupied by each woody species helps understand with some precision the concepts of presence and especially the stability of species. As shown in Table 1, whatever the plant grouping and the bioclimatic stage to which it belongs, the rate of land through all strata combined varies between 45 and 65% but with a minimum of 25% and a maximum of 85% the results that confirm studies by BENABDELI (1996a). These rates are relatively interesting taking into consideration the harsh climatic and anthropogenic conditions. The study of the distribution of occupancy of the soil by stratum and bioclimatic stage will help identify the role of stratification.

The average percentage of land in each stratum is summarized in Table 1.

Whatever the type of plant formation and its dynamics - that arise from work or other kind of pressures exerted on it – it seems that only the forestry groupings of the sub-humid bioclimatic stage offer the best rate of space occupation in all the strata. This is due to the importance of the slice average annual rainfall received and soil quality (soil depth, organic matter content, species composition).

The values obtained confirm the important role of the shrub layers and under shrubs (BENABDELI, 1996b) in the fight against erosion, ~~in~~ the stability of the vegetation cover, in the sustainability and the protection of phytocenosis despite the attacks experienced besides constantly unfavourable environmental conditions affecting 80% of the area for almost nine months out of 12.

The above findings are used to classify the strata according to their importance for the recovery of the global ground. This classification highlights the interest of the physiognomic aspect of forestry groups imposed by the role of each stratum:

Semi-arid Floor	*Subhumid Floor*
1 – Under shrub Stratum	1 - Shrubs Stratum
2 - Shrubs Stratum	2 - Arborescent Stratum
3 – Arborescent Stratum	3 - Under shrub Stratum

The importance of the shrub layer in the semi-arid should be stressed out, which is due to the low covering of the tree layer induced by a low density. The resistance and the power to reject a high percentage (60%) of species in the under shrub stratum makes it possible for the semi-arid to have an occupancy rate of the high space. In the sub-humid bioclimatic stage, the under shrub stratum is almost completely eliminated by the remarkable high rate of soil occupancy of the two other strata. The shrub stratum dominates because individuals composing it are much covering and rapidly growing.

Overall Recovery Rate per Stratum

The following indicator does not overlap with the previous one as it represents the projection of the biomass of all species on the ground and can exceed 100% because there is an overlap between species belonging to different strata. On the other hand, the rate of space occupancy is limited to the land and shall in no event exceed 100%. By stratum or all strata combined, this rate expresses the dominance of a stratum or component species over others. This helps impose a special character in a plant formation based on their fluctuation (DEVAUX, 1976).

The results reveal the dominance of the arborescent stratum in the semi-arid and the shrub stratum in the sub-humid. This is summarised in the following Table 2.

Table 1. Occupancy rate of the soil by stratum and bioclimatic stage

Strata	Semi-arid Stage	Sub-humid Stage
	minimal soil occupation rate	maximal soil occupation rate
Arborescent	17% to 7%	28% to 13%
Shrub	23% to 8%	31% to 23%
Under shrub	25% to 10%	26% to 9%
Total	65% to 25%	85% to 45%

Table 2. Recovering Rate per Stratum

Strata	**Semi-arid Stage**	**Subhumid Stage**	**Mean**
Arborescent	42 to 94%	33 to 97%	65 to 68%
Shrubby	11 to 43%	51 to 127%	45 to 89%
Under Shrub	22 to 67%	7 to 15%	11 to 27%
Total	76 to 204%	91 to 239%	140 to 165%

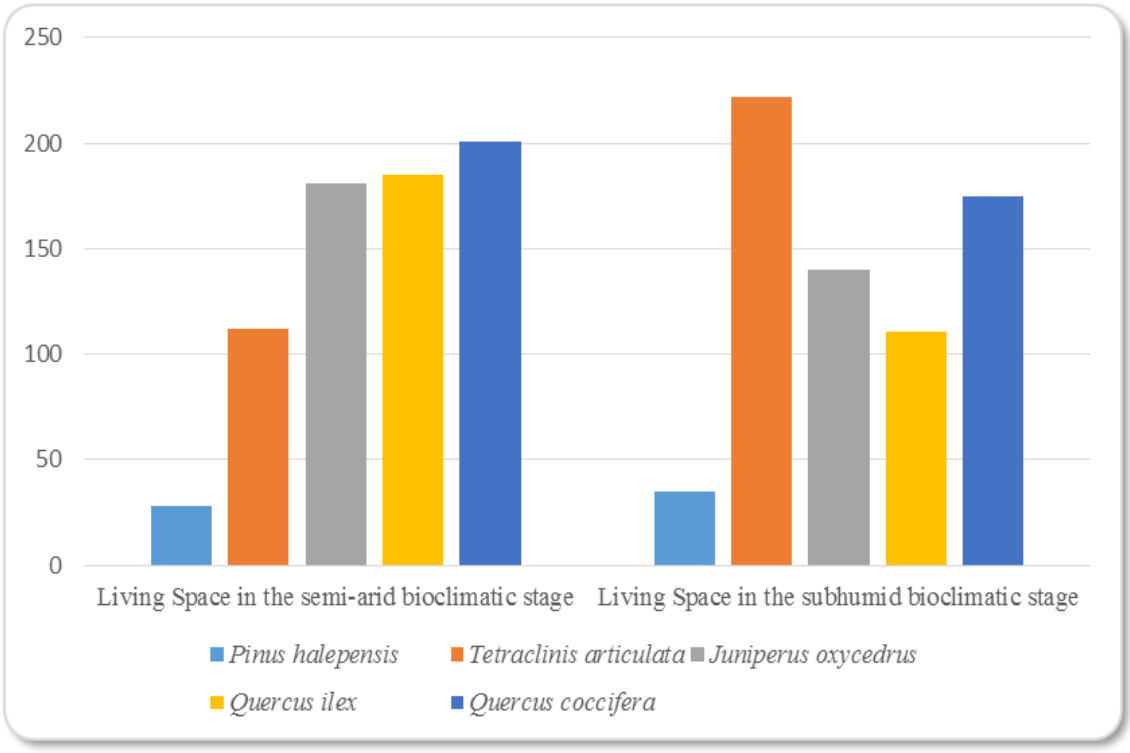

Fig.1. The vital space according to species

Following the importance of the recovering rate, the classification of strata by bioclimatic stage is different as compared to the level of soil occupation. This is summarised below:

Semi-arid Stage
1 - Arborescent Stratum
2 - Under shrub Stratum
3 - Shrubby stratum

Sub-humid Stage
1 - Layer of shrubs
2 - Arborescent Stratum
3 - Under shrub Stratum

In every bioclimatic stage, some strata are dominating and are linked to the role that the level of soil occupation in each stratum plays. Likewise, the floristic composition and its distribution per stratum have an impact on the importance of species with a high recovery rate. The arborescent stratum is important in semi-arid bioclimatic stage whereas in sub-humid stage the shrubby stratum dominates.

The success of restocking and reforestation of degraded forest areas must comply with a choice of species as belonging to a given stratum.

Living Space of the Main Species

The structuring of forest formations usually abides by a ranking of species according to their distribution and to the surface already reserved, or being reserved by man in their silviculture, for each species (PARDE & BOUCHON, 1988). The results help explain the structure and physiognomy of vegetation. In the semi-arid the average area available to each tree is 16 m while it is only 7 m in the sub-humid, this notion of living space varies in the semi-arid from 7 to 25 m² and from 3 to11 m² in sub-humid as well as by individual tree planting stage.

This is an important parameter, all individual young plants have equal opportunities to grow quickly but they compete for space (soil, water and light). The struggle for life will be decisive on any surface. A population grows to the maximum as allowed by the density and by the space left by people who may vacate it.

The average weight of a population of plants would be bound, through a precise mathematical relationship, to their density on a given area.

This is reflected in the work of HARDER (1988) where a line called "thinning right": log W = - 1.5 log d + log k is W = kd-1.3. With W: average weight of dry matter of an individual occupying one square meter (virtual weight of an individual, often those with a circumference of a meter), d: the density per square meter. Only the intensity of light is able to change the position of the right, any change in other factors implies a change of speed in the progression of the right. Thus, the living space is a fundamental that must be mastered to understand the development of individuals of each plant community.

The management of the living space can help control the thinning that enhances the well-planned harvest leading to the development of vegetation formations while increasing the increment of individual plants in diameter and so in volume. The number of subjects to be removed depends on the local ecological potential of gasoline, population age, density in site, and of the targeted objective. DEVAUX'S (1971) definition of the thinning is to make less tight a forest population by removing the subjects from the main species, leaving the trees tight enough to fill the timber and spaced enough for tree to grow well.

The management of this vital space follows some basic parameters which are: the nature, type, the weight, the character, the rotation and the woody material in site. Nature can be quantitative or qualitative. The type consists in the ratio between the volume of the average tree harvested and the volume of the average tree before cutting. The weight is the volume taken from the unit area during a single intervention, that is, the ratio of the volume removed at once and the volume up before intervention. The character includes the nature, weight and type. The cut represents the expression of the treatment. The rotation is the frequency of cuts. The growing stock is the number of bolt according to the dominant height and intensity is the ratio between the average annual volume taken during the cuts and the total annual increase that has maximum volume.

The living space available here is an instrumental value because it allows us to appreciate the potential of the station and provide information on the structure of plant species (THIEBAUT, 1984). The density, which is a reflection of the living space available naturally, or artificially each from individual plant determines all the economic and even ecological aspect of the main plant species.

It provides information on the density, structure and even the physiognomy. The more species have at their disposal a large living space the more a plant shows interesting biometric parameters (height and diameter mostly). DEVAUX *et al*. (1976) who studied the compared structure of Aleppo pine population notes that the living space available to the Aleppo pine averages between 19.7 and 8.2 square meters. PARDE (1988) states that for an optimal timber production, thinning is necessary in

increasing the living space and evaluating the density at 200 subjects per hectare to an age of 70 which is equivalent to 50 square feet per tree. By studying the thinning to be applied in a stand of Aleppo pine in the forest of Nesmoth (Mascara), MAACHOU (1993) recommended for ages 50, 60, 70 and 80 years respectively the densities of 420, 350, 275 and 120 individuals per hectare.

In the area under study, the species of the arborescent stratum have in the semi-arid and sub-humid stage a living space o fluctuating between 28 and 201 m² as confirmed by Table 3, Fig.2. and Fig.3.

Table 3. The vital space according to species.

Species	Living Space in the semi-arid bioclimatic stage	Living Space in the subhumid bioclimatic stage
Pinus halepensis	28	35
Tetraclinis articulate	112	222
Juniperus oxycedrus	181	140
Quercus ilex	185	111
Quercus coccifera	201	175

Fig.2. The vital space for *Pinus halepensis* in the subhumid bioclimatic stage in the forest of Nesmoth (Mascara), (January 2010, Photo KEFIFA)

Fig.3. The vital space for *Pinus halepensis* in the semi-arid bioclimatic stage in the forest of Balloul (Saïda), (April 2011, Photo KEFIFA)

The exploitation of these results confirms the role of recovery rate for each stratum in the choice of living space allowing species to develop and adapt to environmental conditions. The living space of the few species studied remains high in the semi-arid bioclimatic stage, i.e., over 20% in average in relation to the sub-humid floor. These figures may be a reference to justify the choice of density in relation to environmental conditions in the study area for all development operations (planting, restocking and thinning). The optimum densities observed in the different plant formations of the mountains of Dhaya and the mountains of Saida (western Algeria) fluctuate between 160 and 446. This is compared to planting densities in the reforestation and restocking which are currently 2,600 plants per hectare. Lessons are to be drawn as to the high rate of failure of these actions. BENABDELI (1996b) declared in this context that the high densities are the source of competition for pure water resulting in major failures.

These results support the choice of a density linked to the rainfall range in order to ensure sustainability in forestry groupings and in reducing competition.

Conclusion

The importance of controlling the three parameters; land rate, recovery rate and living space (by stratum and by woody species), so often neglected in the descriptt-ion of forest stands, should not be neglectted in the management of forest formations as they play a major role in the dynamics and evolution of forest vegetation.

The study shows also that it is possible through the assessment of these parameters to correct technical errors often made in the choice of species (stratum), planting density and silviculture.

Both the ecological and economic impacts of these guidelines are significant in more than one way and will ensure continuity in forest formations by the application of techniques specific to each type of group.

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Response of Subalpine Saplings to Different Drought Stress

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Abstract. The expectations for increasing periods of drought are becoming larger according to numerous authors. The susceptibility of subalpine tree species to drought provoke our interest to try to understand what will be their reaction to this natural climate change. For this purpose it is set experiment to determine the reaction of drought to 4 subalpine species – Norway spruce (*Picea abies* L.), Mountain pine (*Pinus mugo* Turra), Macedonian pine (*Pinus peuce* Grisebach) and Bosnian pine (*Pinus heldreichii* H . Christ). Different requirements are observed to imitate field conditions as close as possible. The saplings are taken from terrain with no disturbed soil substrate. The plants were placed in a 15 l container and at the beginning of the vegetation were situated in a specially built greenhouse. Precipitation regime is controlled by the irrigation system. The indicators for precipitation levels (for a drought from June to July and August scheme) were taken from the two previous real years, who had a significant influence on the species. Precipitation norm for control is taken from subalpine zone of the Rila Mountain. To determine the reaction of all the groups of saplings subjected to various circuits, at the end of the year is recorded the survivors.

Key words: drought stress, subalpine saplings, precipitation regime, climate change

Introduction

In the recent years, there has been great concern about the future sustainability of subalpine ecosystems in relation to the predicted climate changes (PEREIRA *et al.*, 2006; VINER *et al.*, 2006; CRAWFORD, 2008; LINDNER *et al.*, 2010). The great interest in future responses of these forests on climate change is due to the sensitivity of subalpine tree species to drought (ADAMS & KOLB, 2005), and the high probability of increased frequency and duration of drought in many climate changes scenarios (SHEFFIELD & WOOD, 2007). A voluminous literature emphasizes the effects of environmental stresses on inhibiting plant growth and physiological processes, reducing yield of harvested plant products, and causing plant mortality. Arid conditions, which prevail over about a third of the world's land area, exclude establishment of trees; over most of the remaining land area the growth of plants, and sometimes their survival, is reduced by periodic droughts (KOZLOWSKI & PALLARDY, 2002). Plants differ widely in their capacity to cope with drought. Adaptations exist to explain these differences (PALLARDY, 2008), and these can be conveniently referenced to the capacity to maintain water status (rate of transpiration, stomatal conductance of water vapor and water potential of xylem). LETTS *et al.* (2009) observed the bimodal seasonal pattern of gas exchange in limber pine during the warm and dry growth seasons of 2006 and 2007 would likely not have occurred during cooler and wetter summers. They found that the mortality event occurred during a period

of low precipitation during the wet seasons, which occur before and during the early portion of the growth season. Young trees are more sensitive to the drought boot of their high physiological activity and still not well established mechanisms of drought tolerance (BRÉDA *et al.*, 2006).

The aim of experiment is to determine the survivorship of young trees exposed on different water stress.

Material and Methods

The experiment was conducted in a forest nursery UOGS "G. St. Avramov" – Yundola (1350 m a.s.l.), in a specially built greenhouse, covered with blue panels of plexiglas, who transmit 26% of photosynthetically active radiation (PAR). In this way we imitate natural conditions of light typical of young tree species, who grow in (below the) canopy of a forest. It was found that on the first floor of the forest, the trees absorbed primarily in the red spectrum of PAR (associated with a relatively high amount of chlorophyll in their crowns) and under the canopy of a forest dominated the blue spectrum PAR (PALLARDY, 2008). In this reason were selected blue panels to cover the greenhouse.

In early spring of 2013, from their natural conditions of existence were taken young saplings from four subalpine tree species – Norway spruce (*Picea abies* L.), Mountain pine (*Pinus mugo* Turra), Macedonian pine (*Pinus peuce* Grisebach) and Bosnian pine (*Pinus heldreichii* H. Christ) with no disturbed soil substrate. The plants were placed in a 15 l container and at the beginning of the vegetation, were situated in the greenhouse. For check the survivorship of trees at different levels of soil moisture, the plants were divided into three groups of 40 individuals (10 from each species). To simulate the different circuits of drought was set automatic irrigation system with three rounds of irrigation. In the first of three waters regime (C), the amount of water supplied to the plants was consistent with precipitation norms at subalpine zone of Rila and Pirin Mountains (data from Climate book of Bulgaria). In the second water regime (J) are simulated rainfall amounts by 1993, characterized by drought in June and July. In the third water regime (A) are simulated rainfall amounts since 2000, characterized by drought in the second half of the summer (July-August). Specific precipitation amounts at different irrigation schemes are shown in Table 1.

In the construction of the irrigation system were using electronic programmable controllers to supply the rainfall quantity. Irrigated system were made of ½-inch polyvinyl chloride pipe with nozzle, who flowing 80 ml of water per minute for each container.

The containers were placed on the pallets with a height of 4 cm for separation from the soil moisture. For reducing penetration of sunlight, near 70% from PAR was limited with green screen around the greenhouse. Thus in the greenhouse was achieved relatively constant atmospheric humidity. The dried saplings from each tree species and from each drought regime group were numbered at the ends of July, August and September.

Results and Discussion

The number of dried saplings in July of the four tree species and three rainfall regime are shown in Table 2.

The table shows that most intense was the drying at Mountain pine – 26.7%, followed by Macedonian pine – 16.7% and Bosnian pine – 13.3%. At Norway spruce wasn't observed drying this month. From plants subjected to drought variant "J" dropped expected out most species – 20%, which is explained with the most drying conditions in this scheme of drought. Plants from irrigation scheme "C", dropped out by one species on both Macedonian pine and Bosnian pine, which can be due to stress caused by the transfer from the field.

Unlike July, August drying at the plants was considerably more intense (Table 3).

Drying of Norway spruce was most intense in August – 70% of saplings in a scheme of drought "J" and 80% of saplings in a scheme of drought "A". Intensive drying (by 70%) was observed at the Macedonian pine from the option A and at

the Bosnian pine by option C. The smallest dropping was observed at Mountain pine - only one dry sapling by option A. The most intensive drying in option "A" (40%) and both 22.5% in option "C" and option "J" was observed during this month.

Table 1. Precipitation amounts ($l.m^{-2}$) in different regime of drought

	C	**J**	**A**
June	92.8	27.8	39.8
July	79.2	10.2	13.6
August	56.6	10.2	3.4
September	47.5	81.5	50.9

Table 2. The number of dried saplings in July

Tree species	**C**	**J**	**A**	**Total:**
Picea abies L.	0	0	0	**0**
Pinus peuce Grisebach	1	3	1	**5**
Pinus mugo Turra	1	3	4	**8**
Pinus heldreichii H.Christ	1	2	1	**4**
Total:	**3**	**8**	**6**	**17**

Table 3. The number of dried saplings in August

Tree species	**C**	**J**	**A**	**Total:**
Picea abies L.	0	7	8	**15**
Pinus peuce Grisebach	2	1	7	**10**
Pinus mugo Turra	0	0	1	**1**
Pinus heldreichii H.Christ	7	1	0	**8**
Total:	**9**	**9**	**16**	**34**

Table 4 shows that in September the most intense was the drying at Bosnian pine, which is probably due to the accumulated stress during the summer months. The largest number drop out seedlings from Bosnian pine are in a drought (A), which is explained by the delayed effect, due to low physiological activity. From the other species, Macedonian pine dry intense in September, until from the spruce saplings wasn't observed drying.

At the end of the vegetation, surviving saplings were 54 from the originally planted 120. Distribution by schemes to drought and by tree species is presented in Table 5.

From Table 5 it is apparent that the best survivorship is on Mountain pine (63 %), followed by Norway spruce (50%) and the lowest survivorship have and Bosnian pine and Macedonian pine (33%). This good survivorship of Mountain pine in the investigated schemes of drought confirms the findings of other authors for stronger negative impact on survivorship of this species, to snowless winters than hot summers (BATLLORI *et al.*, 2009). In contrast, other trees that are more affected by summer water stress have also shown mortality peaks in response to summer drought – in the Andes (CUEVAS, 2000), in the Sierra Nevada (LLOYD & GRAUMLICH, 1997) and Tibet Plateau in South China (TAYLOR *et al.*, 2006; ZHAO *et al.*, 2012; ZHAO *et al.*, 2013) found a strong negative impact to summer temperatures.

For Macedonian pine and Bosnian pine PANAYOTOV *et al.* (2010) found negative impact of summer droughts to the width of annual growth rings, which can be

explained by reduced physiological activity under such extreme conditions. SCHULZE *et al.* (2005) however indicate that the strategy for the temporary reduction in the physiological activity of plants prevents death of plants, but this period should not been at the some time with the period of meristem activity.

Table 4. The number of dried saplings in September

Tree species	C	J	A	Total:
Picea abies L.	0	0	0	**0**
Pinus peuce Grisebach	1	3	1	**5**
Pinus mugo Turra	0	2	0	**2**
Pinus heldreichii H.Christ	1	1	6	**8**
Total:	**2**	**6**	**7**	**15**

Table 5. The number of surviving saplings at the end of vegetation

Tree species	C	J	A	Total:
Picea abies L.	10	3	2	**15**
Pinus peuce Grisebach	6	3	1	**10**
Pinus mugo Turra	9	5	5	**19**
Pinus heldreichii H.Christ	1	6	3	**10**
Total:	**26**	**17**	**11**	**54**

Conclusions

Our investigation shows that the best survivorship has Mountain pine. This confirms the findings of other authors for stronger negative impact of snowless winters than hot summers on survivorship of this species. Intensive drying of Norway spruce is due to his biological and ecological characteristics, related with the necessity of protection to mature forest expressed in insurance of more humid air and shaded space in the crown zone, which prevents young trees, from most intensive transpiration in drought period. The effect of stress from moving the Bosnian Pine, coinciding with the period of meristem activity proved irresistible and this is the probable cause of intense drop-out at this species, rather than drought. Mountain pine as hydrophilic, light-demanding and fast growing species appears most sensitive to drought of the studied species.

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Diet of Saker Falcon (Falco cherrug) and Eastern Imperial Eagle (Aquila heliaca) from Central Kazakhstan

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Abstract. We present results from a study on the diet of Saker falcon (n = 15 nests) and Eastern imperial eagle (n = 2 nests) from south Kazakhstan, on the basis of food remains and pellets collected during the 2009 breeding season. The main prey for Saker falcon was predominantly rodents living in middle-size colonies – *Spermophilus erytrogenys* and *Rhombomys opimus*. We also present the results from the diet of two pairs of Eastern imperial eagles nesting close to Balkhash Lake.

Keywords: Diet, *Falco cherrug*, *Aquila heliaca*, Central Kazakhstan

Introduction

The Saker falcon (*Falco cherrug*) and the Eastern imperial eagle (*Aquila heliaca*) have almost overlapping distribution ranges – they are found from central Europe to eastern Asia. Both species have high conservation status: *F. cherrug* is Endangered and *A. heliaca* is Vulnerable, with populations on the decline in recent years (BirdLife International (2014) IUCN Red List for birds).

Until the early 90s of the XX century, the Saker falcon was a common breeding bird throughout its range and it could be found everywhere in Kazakhstan. The number of Sakers decreased during last 20 year on the average by 5–6 times and currently is estimated at about 1000 pairs. The decline has been generally caused by illegal catching of birds for falconry (LEVIN, 2011). On the contrary, *Aquila heliaca*'s population in Kazakhstan stays generally stable to increasing (KARYAKIN *et al*., 2011).

Data on their feeding and diet for both species come predominantly from the northern part of Kazakhstan, such as from the Naurzum reserve, where studies are continuing for over 70 years. Information on the diet of the Saker are provided in VOLOSHIN (1945), GIBET (1960), PERERVA (1979), SOLOMATIN (1974), BRAGIN (1986), PFEFFER (1986), WATSON & CLARKE (2000), BRAGIN (2001), and for the Eastern imperial eagle – GIBET (1960), VARSHAVSKY (1973), SOLOMATIN (1974), KATZNER *et al*. (2006). For the rest of the range of both species the data is limited. This study provides much needed and current data for the diet and the feeding ecology of both species from central Kazakhstan.

Material and Methods

During the breeding season (May–June) of 2009, during monitoring of the population of the Saker falcon (*F. cherrug)* in central Kazakhstan were identified 20 pairs, and

data on diet were collected from 15 nests. Mainly pellets were studies (138), along with a small quantity of food remains. Data on diet were collected from two nests of the Eastern imperial eagle (*A. heliaca*), near the Balkhash lake (Fig. 1). In this part of Kazakhstan both species nest predominantly on poles of the high-voltage electric infrastructure; three nests of the Saker falcon were found on rocks. The Saker utilizes abandoned nests of the Brown-necked Raven (*Corvus ruficollis*) and the Long-legged Buzzard (*Buteo rufinus*).

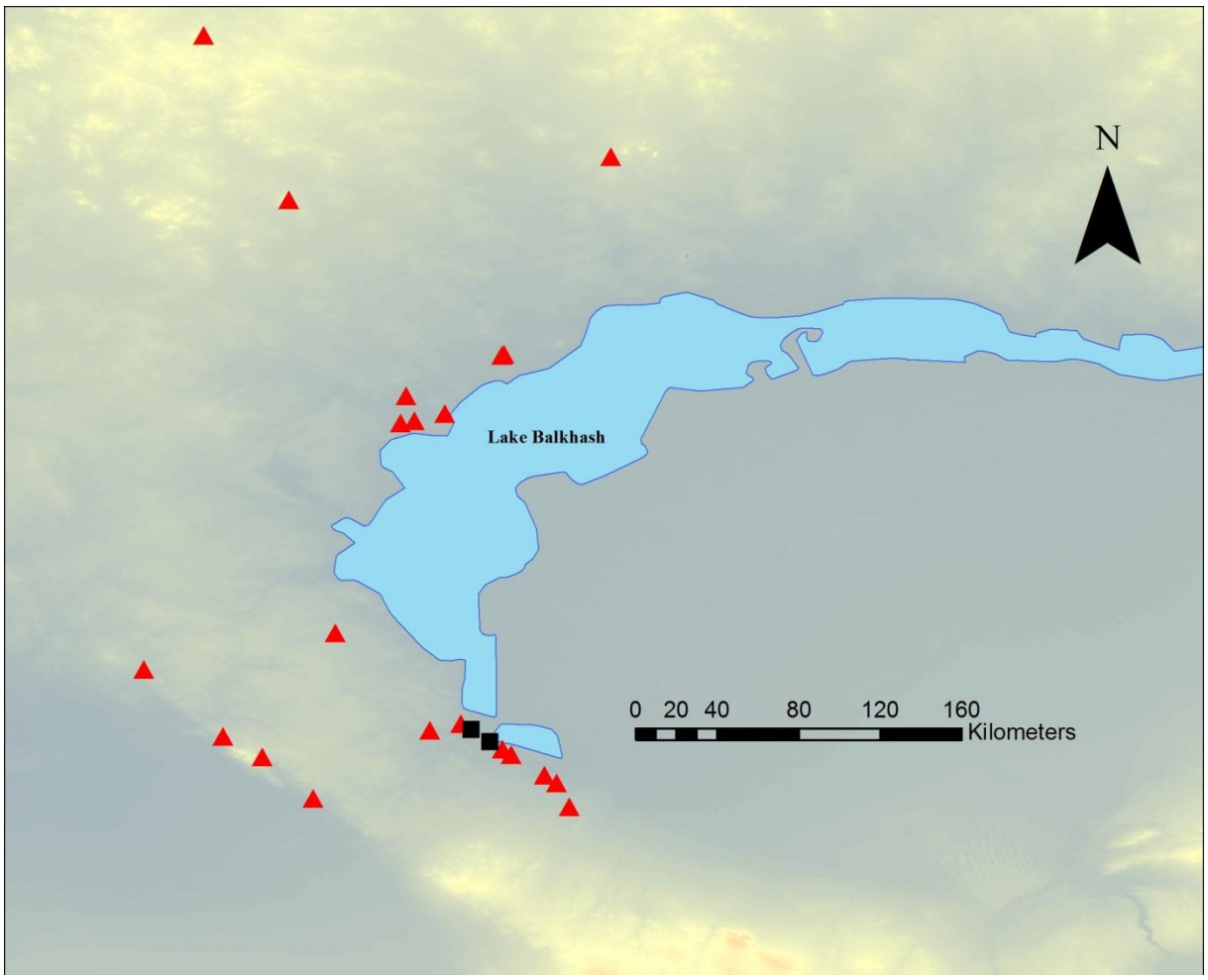

Fig. 1. Study area in central Kazakhstan, near the Balkhash Lake. Triangles show the locations of the 15 nests of the Saker falcon (*Falco cherrug*), squares – the two nests of the Eastern imperial eagles (*Aquila heliaca*).

The materials were collected from the nests or underneath, in a radius of 5–10 meters. The collected pellets were carefully and separately placed in paper and then in bags, to preserve their entirety. Pellet contents and prey remains were identified to the lowest taxonomic level possible based on comparison with reference materials and the minimum number of individuals (MNI) in each pellet or prey remain was estimated from body parts and from quantities of fur or feathers. The mammals were identified based on GROMOV & ERBAJEVA (1995), SLUDSKIY (1978), as well as on a comparative collection of small mammals maintained by NN. Birds were identified based on the comparative osteological collection of the National Museum of Natural History-Sofia, Bulgarian Academy of Sciences.

Study area

The Betpak-Dala desert is situated between the lower courses of the Chu and Sarysu rivers and the western bank of the

Balkhash Lake. The eastern part is a continuation of the Kazakh Uplands (*Kazakhskiy Melkosopochnik*) and a substantial part of it is represented by a rocky desert. The western part is clay plain and is a typical example of a clay desert. The yearly amount of rain is 100–150 mm. The summer is dry and hot, the amount of rain increases from the second half of September. Snow falls from the end of November; it holds for 2.5–3 months (GVOZDETSKIY & MIKHAILOV, 1978).

Several plant associations are found in the desert, the main being *Artemisia terrae albae* – it participates in the formation of almost all plant complexes, *Artemisia sublessingiana* – characteristic of the rocky and clay habitats, *Salsola laricifolia* – the predominant landscape and vegetation cover in the Betpak-Dala desert, *Anabasis salsa* – characteristic of the salinized areas. In the desert, 59 species of mammals have been identified, with 36 of these (63%) being rodents (ISMAGILOV, 1961).

Results

Diet of Saker falcon

In the diet of the Saker falcon were identified 164 food components (Table 1). Mammals occupy the predominant portion (87.8%), followed by birds (9.15%). A major part of the diet is composed of medium-sized colonial rodents – Red-cheeked Souslik (*Spermophilus erythrogenys*) and Great gerbil (*Rhombomys opimus*) form 66.6%. The Gerbils (*Meriones* sp.) also compose an important part of the diet – 10.91%; the rest of the mammals are represented by single individuals. A large portion of the rodents (19.39%) are unidentifiable to the species level because of the poor state of the material in the pellet, but they are from rodents with the size between a Souslik and a Gerbil.

Birds represent a relatively small part of the diet of the Saker (9.15%); the predominant portions were larks (*Melanocorypha, Calandrella*).

Two species of snakes (Serpentes) were also found, but could not be identified to the species level.

Table 1. Diet of the Saker falcon (*Falco cherrug*) from central Kazakhstan, based on 15 nests.

Species	N	N%
Crocidura sp.	1	0.61
Ochotona sp.	1	0.61
Allactaga elater	1	0.61
Allactaga major	1	0.61
Spermophilus erythrogenys	47	28.48
Rhombomys opimus	30	18.18
Meriones lybicus	6	3.64
Meriones meridianus	6	3.64
Meriones sp.	6	3.64
Microtus socialis	2	1.21
Mus musculus	1	0.61
Ellobius talpinus	2	1.21
Rodentia ind. Jerbil/Souslik size	32	19.39
Mammalia ind.	9	5.45
Falco cherrug	1	0.61
Peridix perdix	1	0.61
Coturnix coturnix	1	0.61
Melanocorypha cf. *calandra*	3	1.82
cf. *Melanocorypha*	1	0.61
cf. *Calandrella* sp.	1	0.61
Aves (Passeriformes)	7	4.24
Serpentes	2	1.21
Coleoptera	1	0.61
Ortoptera	2	1.21
Total	**165**	**100.00**

Diet of Eastern imperial eagle

In the pellets and food remains of the two pairs of the Eastern imperial eagles were identified the remains of 33 prey items (Table 2). The diets of the two pairs differ greatly – one pair has specialized in hunting over or near the Balkhash lake. In its diet are prey items, linked with wetland habitats: egrets (*Ardea cinerea, Ardea alba*), Cormorants (*Phalacrocorax carbo)*, and the Muskrat (*Ondatra zibethicus*). The second pair hunts predominantly rodents, with the Great gerbil (*Rh. opimus*) composing 38.1%. Remains of one Horsfield's tortoise (*Testudo* [*Agrionemys*] *horsfieldi*) were also identified.

Table 2. Diet of the Eastern imperial eagle (*Aquila heliaca*) from the region of the Balkhash Lake, based on two nests.

Taxon/Nest	AH1		AH2		Total	
	N	%	N	%	N	%
Spermophilus erythrogenys	1	4.8	1	8.3	2	6.1
Rhombomys opimus	8	38.1	1	8.3	9	27.3
Ondatra zibeticus			4	33.3	4	12.1
Rodentia (Jerbil/Souslik size)	3	14.3	2	16.7	5	15.2
Phalacrocorax carbo			1	8.3	1	3.0
Ardea alba			1	8.3	1	3.0
Ardea cinerea			1	8.3	1	3.0
Aves ind.	8	38.1	1	8.3	9	27.3
Testudo horsfieldi	1	4.8			1	3.0
Total	**21**	100.0	**12**	100.0	**33**	**100**

Discussion

Data from previous studies on the diet of the Saker falcon from the region of Dedpak-Dala are provided by PFEFFER (1986). From the analyzed 54 pellets from three nests, mammals composed the predominant portion of the diet (82.8%), followed by birds (4.7%). The main diet component were Tamarisk jerbil (*Meriones tamariscinus)* (46.9%) and Red-cheeked Souslik (32.8%), comprising 78.7 % of the food of the Sakers. These differences with the data presented in this study might be connected with dynamics in the population abundance, better pronounced with the Gerbils (*Rh. opimus, Meriones sp*.) than with the Souslik (*Spermophilus* sp.). This is mainly due to different climatic factors, as well as to epizootic disease with this group, which serves as a reservoir for plague, tularemia and others (PALVINOV *et al*., 1990; ISMAGILOV, 1961). The abundance of the Great gerbil (*Rh. opimus*) exhibit substantial fluctuations in the periphery of its range, and the Bedpak-Dala desert is in the northern extreme of its distribution (ISMAGILOV, 1961).

Another explanation is that in the desert there is a well-pronounced zonal separation amongst the different rodents. Tamarisk jerbil (*M. tamariscinus*) demonstrates an intra-zonal distribution, inhabiting wetter areas in the desert, in close proximity to water sources – wells, rivers, etc. (ISMAGILOV, 1961). Considering the small sample size of only three nests, it is possible that they were situated in close proximity to a colony of *M. tamariscinus*.

One case of cannibalism was documented. In the nest of a pair with three juveniles an almost complete skeleton of an adult Saker falcon was located. Cases of cannibalism are rare, and so far have been recorded in Kazakhstan (WATSON & CLRACK, 2000) and Slovakia (OBUCH & CHAVKO, 1997).

A predominant part of the diet of the Saker falcon in Kazakhstan is composed of Sousliks (*Spermophilus* sp.). Data from the Naurzum reserve collected systematically since 1936 show an almost doubling in the quantity of Sousliks in the pellets of the Saker (VOLOSHIN, 1946; GIBET, 1960; SOLOMATIN, 1974; BRAGIN 2001).

This is completely different when compared to the situation in central Europe, where the Souslik numbers are decreasing and it has almost disappeared; this prey item has been substituted with pigeons (*Columba* sp.) (FREY & SENN, 1980; BAGYURA *et al*., 1994; HORAK, 1997; OBUCH & CHAVKO, 1997).

Data on the diet of the Eastern imperial eagle are available from the northern part of

Kazakhstan – the Naurzum reserve (SOLOMATIN, 1974). The reserve contains two lakes. The author separates the eagles there in two categories based on diet: "lake" and "steppe" eagles. Some pairs are specialized in hunting birds, connected with wetlands and feeding on aquatic prey such as fish ("lake" eagles), other pairs are specialized in hunting rodents ("steppe" eagles). According to SOLOMATIN (1974), the Eastern imperial eagle hunts predominantly injured or old large waterfowl. Although limited material exists, the two pairs from central Kazakhstan described in this study vary in their diet and each of pairs can be attributed to one of the two categories, proposed by SOLOMATIN (1974). From the desert parts of north of the Aral Sea, VARSHAVSKY (1979) identified the gerbils as a major component of the Eastern imperial eagle's diet.

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A Study on the Effects of Pulp and Paper Industry Sludge on Sweet Corn Crops Grown in Containers

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Abstract. Sludge which are produced from waste-water treatment facilities represent a serious environmental problem in terms of their storage. However, they also represent an organic reserve, biomass rich in macro and micronutrients for soils. Thus, sludge could be used to recover the balance of organic matter in soils. We aimed in the present study to determine the influence of sludge produced from a pulp factory on the development of mays (*Zea mays*) in laboratory conditions.

Keywords: sludge, corn, farming, soil

Introduction

Sludge coming from wastewater treatment facilities is an environmental issue in regard with its production, disposal and reuse. On the other hand, it is an organic soil conditioner, containing high concentration of macro- and microelements (ELMEDIA, 2011). Therefore, sludge could successfully be used for obtaining organic balance in soils (EEA, 2011).

The objective of this research is to estimate the effects of the active sludge produced by pulp and paper industry, wastewater plant on the sweet corn farming in containers.

Materials and Methods

To carry out the experiments an activated sludge material taken from a wastewater treatment plant of a pulp and paper mill was provided. It was previously dewatered on the sludge drying bed next to the plant.

Out coming chemical analyses on the results of the samples was carried out according to the requirements of the Bulgarian Regulation for Sludge treatment, management and utilization in farming, approved with Ministerial Order and issued in State Gazette with Regulation number 339/14.12.2004 - Ordinance Sludge treatment, management and utilization in farming (Ministry of Agriculture and Food, 2004).

The experiments were performed with sweet corn (*Zea mais*) var. "Kneja 613". They were executed in four variants, four times each, with different sample soil.

Each container had capacity of 1 kg.

- Variant I – 100% soil /reference/
- Variant II – 75% soil / 25% sludge
- Variant III – 50% soil / 50% sludge
- Variant IV – 25% soil / 75% sludge

The bottoms of the containers covered with a layer of 100 g felt and after that the mixtures were laid over the felt.

Three grains of sweet corn were planted in every container. They were sowed at depth from 4 to 6 cm. To determine sludge permeability and water retention capacity, the containers were kept moist to 50% and measured every 15 minutes after they had been watered. The weigh measurement showed the following results:

- Variant I – 1.5 kg
- Variant II – 1.4 kg
- Variant III – 1.35 kg
- Variant IV – 1.25 kg
- Variant V – 1.15 kg

The corn samples were planted in a greenhouse on 1st May, 2013. They grew under well-controlled environmental conditions: optimal humidity and ambient temperature between 27-28°C. The moisture was kept at a constant level by regular watering up to the original weight.

The following processes were monitored: germination, first and second leaf growth, third and fourth leaf growth and the stage of fifth and sixth leaf growth.

The experimental soil was put to analysis in Agricultural Chemistry and Soil Science Department at Agricultural University of Plovdiv. The following analysis methods were applied to it:

- *Mechanical composition analysis by means of FRITISH vibratory sieve shaker*
- *pH in H_2O – potentiometric pH measurement*
- *Humus content determination by the method of Turin*
- *K content was determined in salt- acid based extraction of 2n HCl*
- *Movable phosphates were determined by Egner-Riem method (DL-method)*
- *Ammonium and nitrate N in an extract of 1% KCl*
- *Determining carbonate concentration level by Schibler method*

Results and Discussion

Parameters of activated sludge are presented in Table 1.

Table 1. Chemical analysis of activated sludge

Parameter	Value
pH	8.07
Organic substance	71.69%
Dry solid	42.58%
Escherichia coli	0.1 g
Salmonella spp.	lack
Clostridium perfringens	0.001 g
Cd	0.46 mg kg^{-1} dry solid
Cu	11.6 mg kg^{-1} dry solid
Ni	6.13 mg kg^{-1} dry solid
Pb	6.15 mg kg^{-1} dry solid
Zn	50 mg kg^{-1} dry solid
Hg	<0.05 mg kg^{-1} dry solid
Cr	7.46 mg kg^{-1} dry solid
As	<0.05 mg kg^{-1} dry solid
Nitrogen /total/	11.38 mg kg^{-1} dry solid
Phosphor /P_2O_5/	1341 mg kg^{-1} dry solid
Potassium /total K_2O/	340 mg kg^{-1} dry solid
PAH /polycyclic aromatic hydrocarbon/	<0.01 mg kg^{-1} dry solid
PCB /polybrominated biphenyl/	<0.005 mg kg^{-1} dry solid

During the preparation of the experimental variants, and namely soil, sludge and soil/ sludge ratio, it was observed that the sludge featured bigger volume and took bigger space in the containers. It also had less density and featured better permeability than the soil and better aeration. Although the sludge was more permeable, it retained the moist longer.

Table 2. Physical and chemical properties

Moisture, %	Humus, %	Total Nitrogen, %	$CaCO_3$, %	pH in H_2O
4.48	1.7	0.25	7.48	7.8

Table 3. Agrochemical parameters

Soil Type	Depth, cm	Min. Nitrogen $NH_4 + NO_3$ mg kg^{-1}	P_2O_3 mg per100g	K_2O mg per100g
Alluvial meadow soil	A_I 0 – 10	13.4	12.4	26

According to the World Soil Classification developed by FAO alluvial meadow soils relate to molikovite fluvisoles class.

Alluvial soils are soils deposited by running water. They feature well-formed humus accumulative horizon, which gradually transforms to C – type horizon.

These types of soil are widely spread in Bulgaria. They are located in the central part of river valleys right after the alluvial meadow soils towards the first land off shore terrace. Such soils exist also at the first low off shore terrace. They are divided in two types: calcic and non – calcic ones. Further they are classified according to the depth of soil graying (swallow – 100-150 cm, average deep – 150-200 cm, deep – bellow 200 cm.)

Humus horizon as well as the fixed soil layers is colored in brown with different intensity of the color shades. The humus horizon is about 30-40 cm high.

The examined soils had an average sandy – clay composition. They featured high permeability, average water retaining capacity and relatively good aeration capacity.

Humus content in the upper horizon was 1.7 %. Carbonates have been detected at the surface. Their availability explained the slight alkaline reaction (see Table 1) – pH=7.8.

Regarding the microelements, their content in the examined soil samples was evaluated as poor – regarding the nitrogen, as average – regarding the phosphor, and good – regarding the potassium. (see Table 2)

They are considered being beneficial in regard with their physical and chemical qualities and are the most fertile soils in the municipality land. Being friable and not very flexible or clayey, these soils show slight resistance when processing. They are suitable for farming serials.

The seeds sprouted on the sixth day after their sowing (Table 4)

Table 4. Seed germination

Variant	Number of containers	Number of sprouts	% sprouts
Variant I	4	11	92
Variant II	4	12	100
Variant III	4	8	67
Variant IV	4	3	25
Variant V	4	2	17

The results in the table show that the biggest number of sprouts was observed at Variant II, followed by Variant III. The experimental Variants IV and V obtained little number of sprouts. Therefore, it can be

concluded that the increasing the sludge content in soil delays the germination/sprouting.

Next table shows the records made on 9th May, 2013 when the appearance of 1st and 2nd leaves was observed (Table 5).

Table 5. Number of sprouts at the 9th day of the experiment

Variant	Number of containers	Number of sprouts
Variant I	4	12
Variant II	4	12
Variant III	4	12
Variant IV	1 1 2	2 1 0
Variant V	2 1 1	4 1 0

As the table shows, the results in Variant IV remained (See Tables 4 and 5).

Observation on the 3rd and 4th sprout stage was recorded on 26th May, 2013 and the results are shown on Table 6.

The report shows that all the sprouts in the soil, which was used as a reference, were in a stage of the 4th leaf. In Variant II, there were plants in 5th leaf stage. In Variant III we observed massive germination of 5th leaf. There were least number of sprouts in Variant V, however the sixth leaf of the plants was best formed.

Table 6. Number of sprouts at the 25th day of the experiment

Variant	Number of plants	Stage of growth
Variant I	All the plants are in stage of 4th leaf	
Variant II	10 2	4th leaf stage 5th leaf stage
Variant III	All the plants are in stage of 5th leaf	
Variant IV	1 2	4th leaf stage 5th leaf
Variant V	1 1 1	4th leaf stage 5th leaf stage 6th leaf stage

The last record on the growth stages was done on 30th May, 2013. The records showed that most of the plants were in their stage of development 5th-6th leaf. More precisely: Variants I and II had plants in 5th leaf stage; Variants IV and V – plants in 6th leaf stage, respectively.

At true leaf stage we carried out measurements of the plants height, stems length and thickness (Fig. 1).

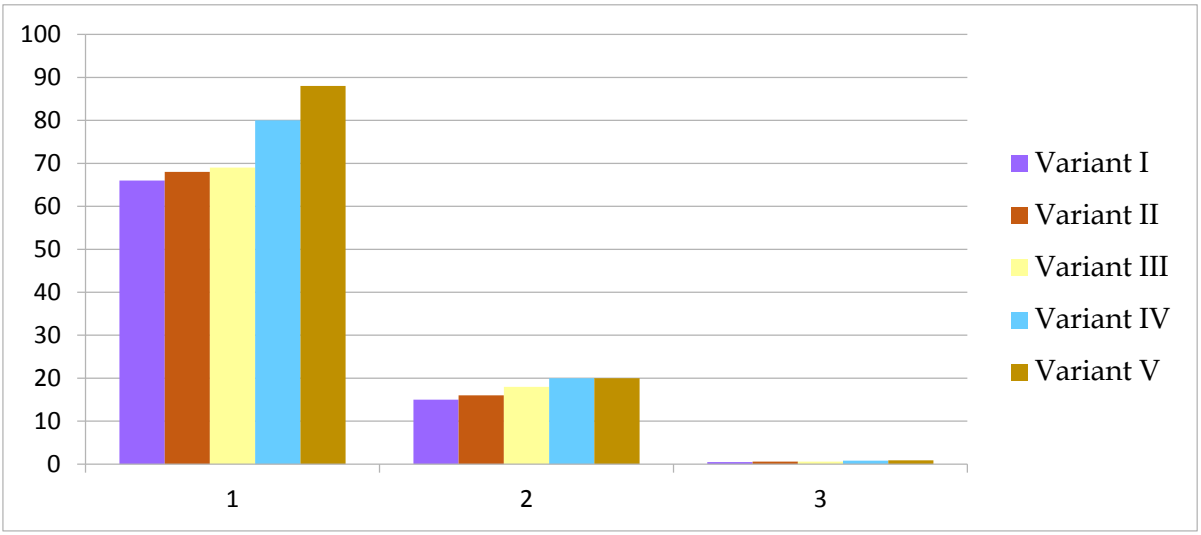

Fig.1. Biometrical data about the plants grown in containers (cm)

The records show that the plants in Variants IV and V obtained the most favorable characteristics. They stood out from the rest of the variants for having the highest plants with the longest and thickest stems.

From the obtained results, it was concluded that the plants, which grew in soils with greater sludge content, grew faster, had greater biomass and much better developed root system. The root system development was proportional to the sludge proportion in the soil.

Conclusions

As a results of the experiment carried out with corn seeds of sort "Kneja 613", grown in a soil and active sludge with content ratio: 3:1; 1:1; 1:3 and 100% sludge, it was concluded that:

1. Plants in Variant II and III grow earlier in comparison with the rest of variants, however Variants IV and V has faster growth.

2. At the time of growth assessment of 3rd – 4th leaf stages, Variant III showed massive appearance of fifth leaf. Variant V showed least number of sprouts; however, the same plants had the best-formed sixth leaf.

3. With best plant development/ growth are Variants IV and IV. They stand out from the rest of the Variants for having the highest plants with the longest and thickest stems.

4. Plants, which grow in soils with greater sludge content, grow faster, have greater biomass and much better developed root system. Root system development is proportional to the sludge proportion in the soil.

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Genetic Differentiation between Mullus barbatus from the Western Part of the Black Sea and Mullus surmuletus (Pisces, Mullidae) from the Mediterranean Sea

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Abstract. Genetic divergence and phylogenetic relationship of two species *Mullus barbatus* from the western part of Black Sea (Varna) and *M. surmuletus* from the Mediterranean Sea (Thessaloniki) were investigated using the electrophoretic data from enzymatic systems, codifying for 15 putative loci, and the patterns of general muscle proteins (PROT) coded from nine loci. Several loci *PROT-4*, PROT-5** and *PROT-8** as well as two *mMDH* and two *sMDH* loci, and *LDH-A** showed different electrophoretic patterns among species and can be used as species-specific markers. Only one esterase locus (*EST-9**) was found to be polymorphic for both species. The remaining enzymes and proteins were monomorphic. In this study for the first time existence of hybrids between two species were reported. Hybrids were registered in the Mediterranean Sea (Thessaloniki) as well in the northeastern part of Black Sea (Balshoj Utrish) using electrophoresis and isoelectric focusing methods. Genetic distance D_{Nei} (0.526) and time of divergence (t_{Nei} = 3 215 000 years) between *M. barbatus* (Varna Bay) and *M. surmuletus* (Thessaloniki) give evidence for existence of these two well diverged species in one genus.

Key words: genetic divergence, phylogenetic relationship, *Mullus barbatus, Mullus surmuletus,* Black Sea, Mediterranean Sea.

Introduction

The genus *Mullus* is represented by two species - *M. barbatus* and *Mullus surmuletus*.

M. barbatus L. 1758 is distributed throughout the Mediterranean Sea, as well as in the eastern Atlantic, from the British Islands in the north to Senegal in the south (HUREAU, 1986, cited after TURAN, 2006). *Mullus barbatus* in the Black Sea is taxonomically classified as a subspecies *M. barbatus ponticus* Essipov, 1927 (KARAPETKOVA & ZHIVKOV, 2006; TURAN, 2006).

KESKIN & CAN (2009) and VASILJEVA (2012) on the base of molecular, morphological and karyological data have not verified the existence of *M .barbatus ponticus* as a subspecies.

M. surmuletus L., 1758 is distributed throughout the Mediterranean Sea, in the Atlantic, from Norway to the Canary Islands, in the Black Sea and in the northwestern coasts of Africa. VASILJEVA (2007) pointed that this species is registered only along Turkish coast of the Black Sea.

Some studies analysed the genetic variation in *M. barbatus* and *Mullus surmuletus*, identifying diagnostic loci between the two species using allozymes (BASAGLIA & CALLEGARINI, 1988; CAMMARATA *et al*., 1991; MAMURIS *et al*., 1998, 1999). ARCULEO *et al*. 1999; TURAN, 2006). MAMURIS *et al*. (1998) suggested that allozyme analysis provide important information of the genetic structure of the red mullet to ensure sustainable management of this species. MAMURIS *et al*. (2001) and APOSTOLIDIS *et al*. (2001) investigated genetic structure of *M. barbatus* and *Mullus surmuletus* in the Mediterranean Sea, by means of RFLP analysis of PCR-amplified mitochondrial DNA.

According to VASILJEVA (2012) the level of genetic divergence between Mediterranean and Black Sea red mullets is not defined.

The main **goals** of this study were to find diagnostic loci between *M. barbatus* from Bulgarian Black Sea coast and *M. surmuletus* from Mediterranean, to assign the genetic distances between them as well as to find interspecies hybrids.

Material and Methods

54 fish *M. barbatus* from the Black Sea (Varna Bay), 10 fishes from Bolshoj Utrish (Russia) and 15 fish *Mullus surmuletus* from Mediterranean Sea (Thessaloniki) were collected from 1993 -2010 (Fig.1.)

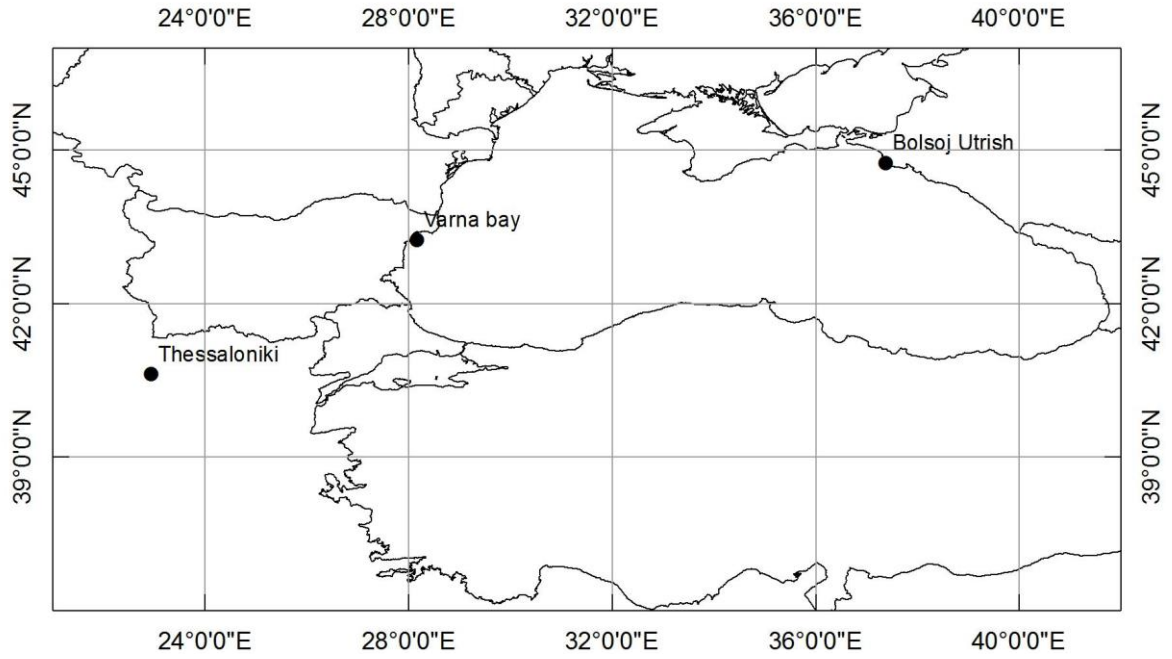

Fig.1. Map of sampling locations for Mullidae species.

For the analysis of the enzymes and non-enzyme protein systems, a homogenate of white dorsal muscle was used. Proteins were separated by horizontal starch gel electrophoresis according to SMITHIES (1955) methods, modified by DOBROVOLOV (1973). Isoelectric focusing (IEF) on thin polyacrilamide Ampholine gel with pH gradients between 3.5-10.0 was applied, as well as IEF on ultra-thin polyacrylamide Servalyte gel plates provided by LKB (Stockholm, Sweden). The proteins were stained with Commassie Brilliant Blue R-250. Staining of different enzymes was performed according to Shaw and Prasad (1970). Buffer systems of DOBROVOLOV (1976) and CLAYTON & GEE (1969) were used for the electrophoresis. The following enzymatic systems were studied: esterase (EC 3.1.1.1 - EST), lactate dehydrogenase (EC 1.1.1.27 - LDH) and malate dehydrogenase (EC 1.1.1.37 – MDH).The nomenclature of mentioned loci and alleles followed essentially the recommendation of SHAKLEE *et al*. (1990). Gene frequencies of the polymorphic loci were calculated using

the Hardy-Weinberg equilibrium. Calculation of indices of genetic similarity and genetic distance was performed according to NEI (1972).

Results and Discussion

General muscle proteins (PROT) **-** The general muscle protein fractions (PROT) on isoelectric focusing (IEF) on ultra thin gel plate as well as on starch gel electrophoresis (Fig. 2 and 3) of the examined mullid species demonstrated differences on the species level. Nine loci on general muscle proteins were analyzed (Fig.3) and the difference between the species were presented. The data received, support the CAMMARATA *et al*. (1991) evidence for existence of species-specific patterns on general muscle proteins on the two species compared. The *M. barbatus* samples, caught at different localities of the Black Sea (Varna and Boljshoj Utrish) have equal electrophoretic patterns (Fig. 2 and 3). On Figure 2 one of the samples (N2) showed spectra, typical for the hybrids between different species. Obviously in the Mediterranean Sea the hybrids between *M. barbatus* and *Mullus surmuletus* is registered. Hybrids between two species were found also in Balshoj Utrish (north-eastern part of the Black Sea, Fig.3, N13-15). All analyzed samples from this area showed the same hybrid spectra.

In the Black Sea three allelic type of inheriting of *EST-9** locus (Fig.4, Table 1), while in Mediterranean two allelic polymorphism on this locus were observed. Other analyzed esterase loci were monomorphic. Species specific electrophoretical spectera on *EST-2**, *EST-7**and *EST-8** of *Mullus surmuletus* and *M. barbatus* were were observed (Table 1).

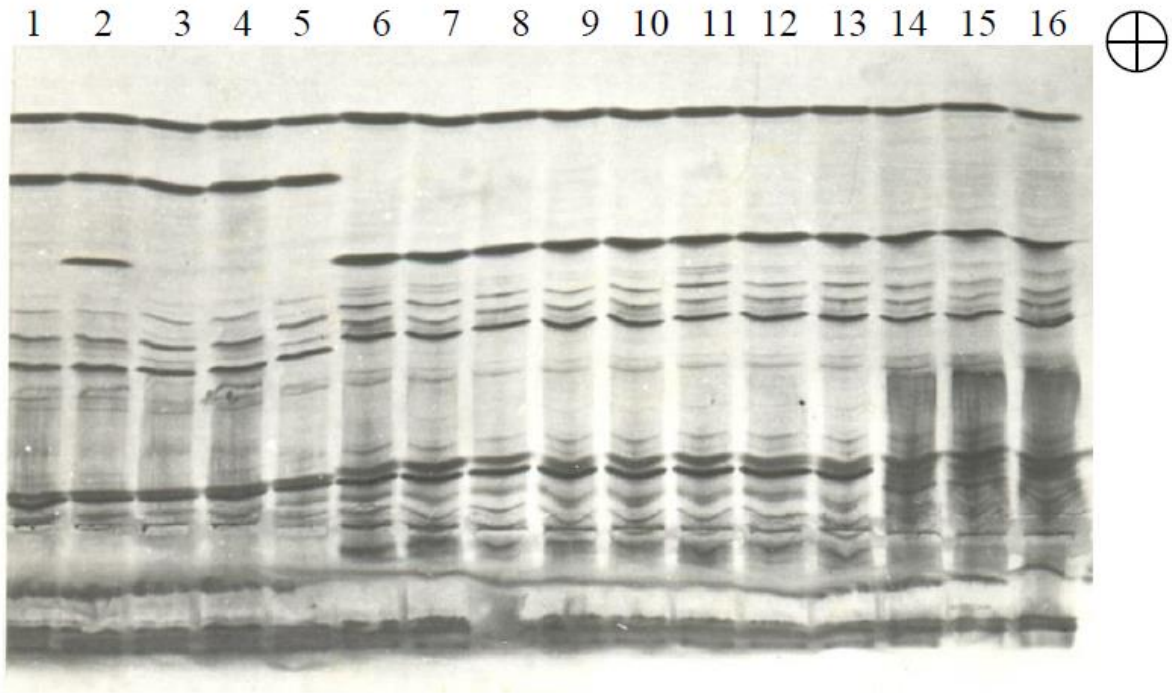

Fig. 2. Isoelectric focusing (IEF) on ultrathin polyacrilamide Ampholine gel plate with pH range 3-10: 1-5 - *Mullus surmuletus*, Mediterranean Sea, 6-13 - *M. barbatus*, Varna Bay, Black Sea, 14-16 *M. barbatus,* Balshoj Utrish, Russia.

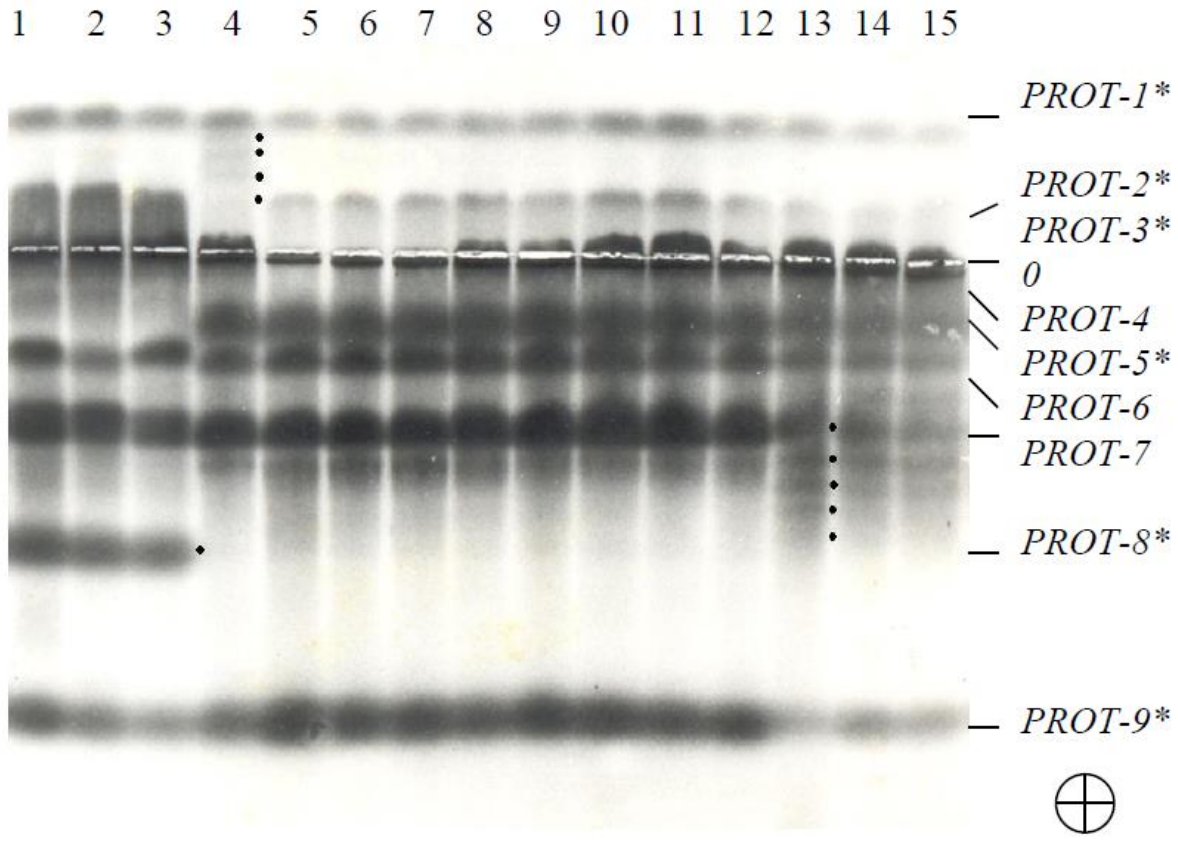

Fig.3. Electrophoregrams on general muscle proteins (PROT) : 1-3 - *Mullus surmuletus*, Mediterranean Sea, 4-12 - *M. barbatus* , Varna Bay, Black Sea, 13-15- *M. barbatus,* Balshoj Utrish, Russia, showed hybrid spectra, marked with dots, 0 – origin.

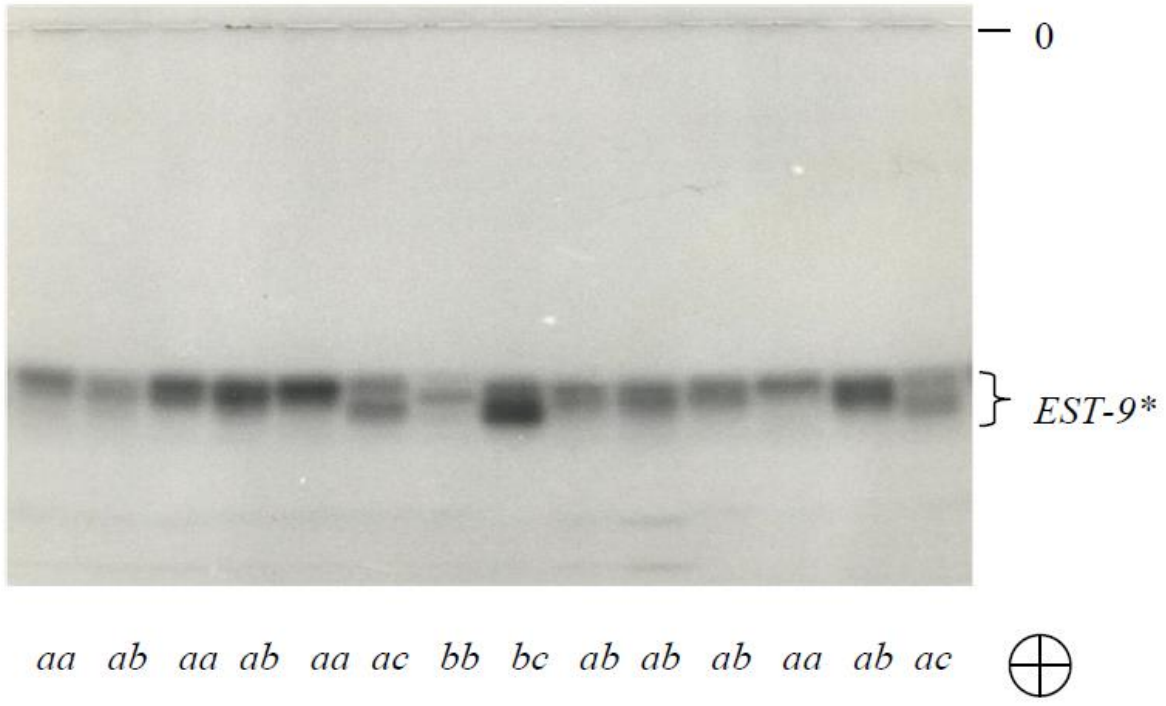

Fig.4. Electrophoregrams of esterases (EST) on starch gel on *Mullus barbatus,* Varna Bay. Polymorphism with three allelic type of co-dominant inheriting was registered. aa, ab and bb – phenotypes. O – origin.

Except esterases, the remained species specific enzyme systems which have been analyzed occurred to be monomorphic (Table1).

Concerning lactate dehydrogenase, two loci (*LDH-A** and *LDH-B**) were visualized on the electrophoregrams. According to DOBROVOLOV (1996) the species, which belong to the same genus have equal *LDH-B** position. The genus specific *LDH-B** spectra of red mulled and striped mulled has also equal electrophoretic mobility. The observed lack of differences at the locus *LDH-B** between two species is consistent with the results obtained by CAMMARATA *et al.* (1991) and MAMURIS *et al.* (1998). *LDH-A** locus was monomorphic with different electrophoretic mobility by two species compared. This locus is species specific (Fig.5).

Four malate dehydrogenase loci were monomorphic in the investigated species. Two *sMDH* loci and two *mMDH* loci were observed with species specific differences on the both species (Fig.6). The samples from Balshoj Utrish showed hybrid spectra on this enzyme system.

The hybrids have fractions of two species analyzed.

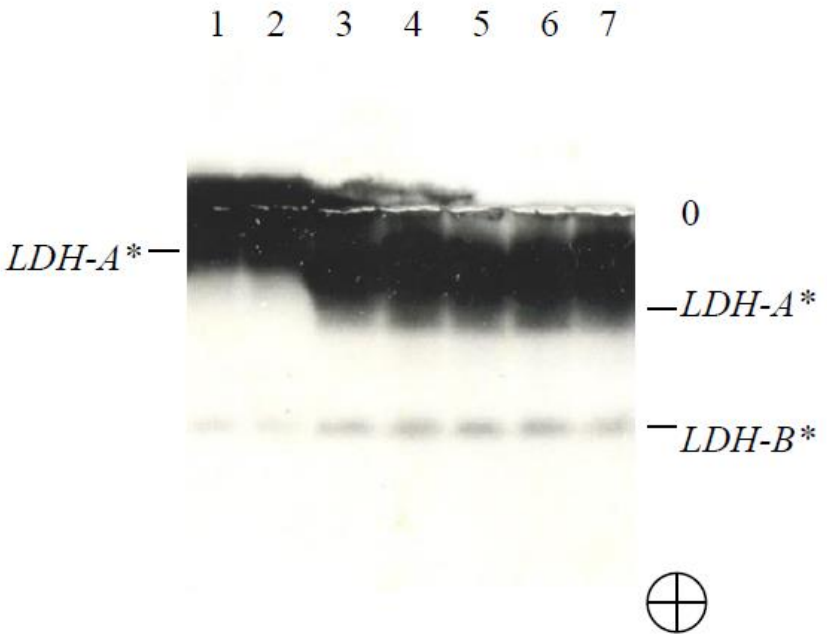

Fig.5. Zymograms of lactate dehydrogenase on starch gel: 1-2 - *Mullus surmuletus*, Mediterranean Sea, 3 – 5 - *M. barbatus*, Varna Bay, Black Sea, 6-7 *M. barbatus,* Balshoj Utrish, Russia

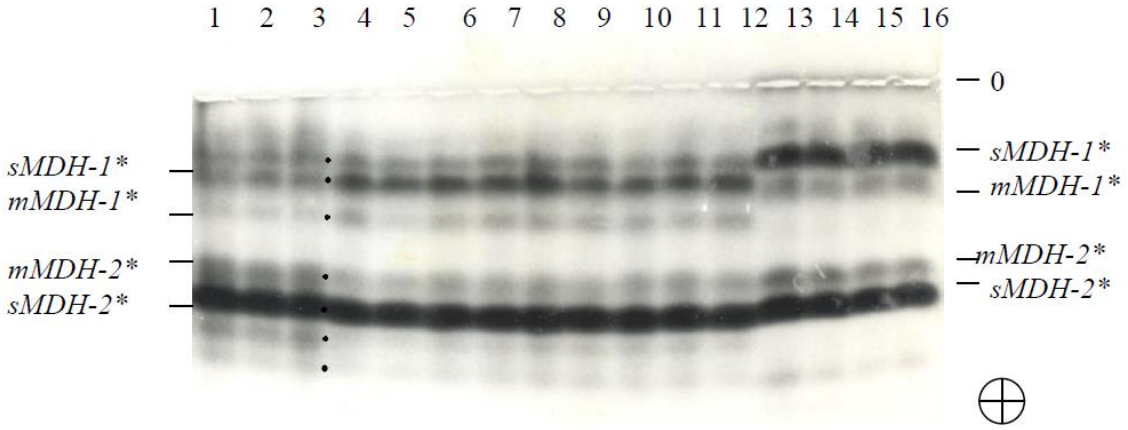

Fig.6. Zymogram of malate dehydrogendase (MDH) on starch gel:1-3 *M. barbatus,* Balshoj Utrish, Russia (hybrid spectrum was marked with dots), 4-12 - *M. barbatus* , Varna Bay, Black Sea, 13-16 - *Mullus surmuletus*, Mediterranean Sea, 0-origin.

Table 1. Genetic distance (D_{Nei}) between species *M. barbatus* (Black Sea, Varna Bay) and *Mullus surmuletus* (Mediterranean Sea, Thessaloniki), calculated on the base of 9 protein and 15 enzymic loci.

Species Alele	*M. barbatus* Black Sea	*Mullus surmuletus* Mediterranean Sea	D_{Nei}
*EST-1**	1	1	1
*EST-2**	0	1	0
*EST-3**	1	1	1
*EST-4**	1	1	1
*EST-5**	1	1	1
*EST-6**	1	1	1
*EST-7**	0	1	0
*EST-8**	1	0	0
*EST-9**	a-0.544 b-0.324 c-0.132	a-0.125 b-0.875 c-0	0.615
*PROT-1**	1	1	1
*PROT-2**	1	1	1
*PROT-3**	1	1	1
*PROT-4**	0	1	0
*PROT-5**	1	0	0
*PROT-6**	1	1	1
*PROT-7**	1	1	1
*PROT-8**	0	1	0
*PROT-9**	1	1	1
*LDH-A**	1	0	0
*LDH-B**	1	1	1
*sMDH-1**	0	1	0
*sMDH-2**	1	0	0
*mMDH-1**	0	1	0
*mMDH-2**	1	0	0
D_{Nei}			0.526

Genetic distance D_{Nei} (0.526) and time of divergence (t_{Nei} = 3 215 000 years) between *M. barbatus* from the Black Sea and *M. surmuletus* from Mediterranean, calculated on the base of 24 analyzed loci, give evidence for existence of these two well divergated species in one genus.

The Nei's genetic distance presented is more close to this (D=0.329), calculated from MAMURIS *et al*. (1998) between the two species *M. barbatus* and *M. surmuletus* in Mediterranean Sea. The higher genetic distance calculated by us could be result form the comparison of two species, inhabited two basins (Black Sea and Mediterranean Sea). We did not support CAMMARATA *et al*. (1991) opinion for high similarity between the two *Mullus* species (D =0.068).

Allozyme data of analyzed samples from Balshoj Utrish showed that they are 100% hybrids between *M. barbatus* and *M. surmuletus* and prove the existence of the *M. surmuletus* species along the Russia coast.

One morphological parameter - standard length (SL) measured for the *M. surmuletus* from Thessaloniki (Mediterranean Sea) varied from 16 to 20 cm, for the *M. barbatus* from Varna Bay (Black Sea) – between 10.7 and 15.6, while of the hybrid samples from Bolshoj Utrish have intermediate values from 14.5 to 17.5cm.

Conclusions

General muscle proteins and enzymes analysed could be used as species specific markers.

The genetic distance, calculated on the basis of alloszymes between *M. barbatus* and *M. surmuletus* (D=0.526) and time of divergence (t_{Nei} = 3 215 000 years) give evidence for existence of two well divergated species in one Genus.

For the first time hybrids between *M. barbatus* and *M. surmuletus* in Mediterranean and Black Sea (Boljshoj Utrish, Russia) were registered using two electrophoretic methods.

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Natural Plant Oils and Terpenes as Protector for the Potato Tubers against Phthorimaea operculella Infestation by Different Application Methods

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Abstract. For protecting potato tubers from the potato tuber moth (PTM) infestation during storage, different concentrations of ten natural plant oils and three commercial monoterpnes were tested, some as fumigants or dusts against adults or dusts against neonate larvae, while others as sprays on the gunny sacks in which potato tubers were stored. Tuber damage indices as well as persistence indices for tested materials were assessed. Vapors of *Cymbopogon citratus, Myristica fragrans* (nutmag), *Mentha citrata and a-Ionone* (monoterpene) caused a highly significant reductions in the life span of exposed moths as well as in new adult offsprings. Other tested oils as *Cinnamonium zeylanicum, Myristica. fragrans* (Mace) and *Pelargonium graveolens* caused a insignificant effect. There was no significant effect of the tested vapors on egg hatchability, except in case of oils of *C. citratus, M. fragrans* (nutmag) and *M. tragrans*(Mace oil) which caused high reduction in egg hatchability. According to the values of damage indices, the most effective oil vapors were arranged ascendingly as follows: *Myristica* (nutmag) < *Cymbopogon* < *Mentha* < *a - Ionone.* Dusting potato tubers with 1% conc., (mixed with talcum powder) of *Myristica, Mentha, Cymbopogons* oils and a-Ionone (monoterpene) caused high reduction in egg deposition, adult emergence as well as percentage of penetrated larvae of PTM. According to their damage indices, *Cymbopogon* and ά-Ionone were the most protective oils, followed by *Myristica* and *Mentha.* Spraying gunnysacks with 1% conc., of the aforementioned natural oils separately elicited high reduction in PTM progeny; while their combinations did not elicit any significant synergistic effect. According to their tuber damage indices, it was found that *Cymbopogon* oil alone or mixed with *Myristica* oil showed the best protective effect, followed by *Myristica* oil alone and *Mentha* oil mixed with *Cymbopogon* oil. Assessment of the persistence index of various tested materials during storage indicated that: Vapors of *Cymbopogon* and *Myristica* (nutmag) gave the best protection from PTM infestation during storage (for 20 days). Dusting potato tubers with plant oil of *Cymbopogon* (mixed with talcum powder) gave the best protection during storage (for 15 days). Spraying gunnysacks with natural plant oils of *Cymbopogon* and *Myristica,* separately or mixed elicited the best protection from infestation by PTM during storage up to 20 days.

Key words: Potato tuber moth, *Phthorimaea operculella*, Natural oiles, Terpenes, Different application treatments.

Introduction

There is a renewed interest amongst scientists to study the bioactivity of plant essential oils and their constituents of terpenes against phytophagous insect pests (BAKKALI *et al*., 2008, ADORJAN & BUCHBAUER, 2010). The antifeedant and repellent effects of various plant essential

oils have been reported on weevils *Sitophilus granarius* L. and *Sitophelus zeamaiz* Mostschulsky (Coleoptera Dryophthoridae), both widespread stored grain pests (CONTI *et al*., 2010, 2011; MOSSI *et al*., 2011).

In developing countries, most of the potato farmers cannot afford the increasing cost of storing potatoes in cold stores, so the tubers are often heaped under a tree or in rustic shelter and covered with a thick layer of straw, potato foliage or another handy materials (ESSAMET *et al*., 1988). Other farmers rely heavily on insecticides, which are dusted or sprayed on the tubers at the beginning of the storage period. However, the prophylactic use of insecticides, especially for stored potato tubers, causes serious problems. These include the development of insect resistance to insecticides, persistence of residues in tubers, destruction of beneficial organisms, human intoxication and contamination of the environment. Recently, several programs for pest control have been developed, including the use of powders and oils of natural plant origin, resistant potato strains (varieties), or using intercropping system, etc. SHELKE *et al*. (1985) studied the effect of seven vegetable oils against adults of the PTM on potato tubers in the laboratory. They found that 0.05 and 0.1 % neem oil *(Azadirachta indica)* had Oviposition deterrent effect. SHARABY (1988) mentioned that reproduction in the potato tuber moth was significantly reduced when either males or females were exposed to the vapor of orange peel oil and such effect increased with increasing oil concentration and exposure time. In addition, a pronounced reduction in egg production and egg viability occurred when the moths were exposed to the vapors arising from oil treated paper discs. EL-NAHAL *et al*. (1989) tested the toxic effect of the vapors of the essential oil *Acorus calamas* (L.) rhizomes on the adults of several stored grains. HOOSHANG *et al*. (2013) investigated the fumigant toxicity of essential oils of Basil, European pennyroyal, Lavender, Mint and Savory, on potato tuber moth. Treated Potato tubers by methanoilc extract of Lavender elicited the lowest percentage of first larval penetration. Probit analysis of essential oils showed that the most effective oil was Savory oil. SHARABY *et al*. (2014), recorded that Dusting potato tubers with bulb powder of *Allium cepa* (50% cone. mixed with talcum powder) displayed a highly effective role in the reduction of deposited eggs as well as adult emergence there from. *Allium cepa*, *Pelargonium graveolens* and *Cymbopogon citratus* oils caused high reduction in larval penetration into treated tubers. Mixture of *Pelargonium*or *Allium* mixed with talcum powder gave good protection for a long storage period (30-40 days). The aim of the present work is to studying the effect of various plant oils and commercial monoterpenes, for protecting the potato tubers from PTM infestation during storage period.

Materials and Methods

A stock laboratory colony of the PTM has been raised on potato tubers, which are the main natural host. The culture was maintained under laboratory conditions at 26 ± 2°C and 70 ± 5 %R.H.; following the technique mentioned by EL-SHERIF (1966).

Protection of potato tubers from PTM infestation during storage

A - Fumigation.

a- Treatment of adults

Virgin male and female moths were continuously exposed to vapors of ten natural oils and three commercial monoterpenes. The tested oils were Bergamote, Lemongrass, Mace, Nutmag, Clove, Eucalyptus, Ginger grass, Cinnamone, Cedar wood and Geranium (Table 1). They were obtained in pure state from the "Sugar and Complete Industrial Company" El Ahram Street - Giza. The commercial monoterpenes were Geraniol, a-Ionone and IsoeugenoL. They were obtained from Sigma Aldrich Corporation, U.S.A. A quantity of 0.05 ml of the tested oil was introduced into a small plastic tube (1 x 3 cm) that was suspended by a thread from the lid of a test container (500 ml). Each container was provided with 2-3 clean tubers weighing about100gm. The following aspects were determined: (1) Longevity of

treated male and female moths. (2) Number of emerged offspring. (3) Damage index of tuber: The assessment of the mean index of damage was determined according to the following categories based on the degree of larval tunneling visible from external examination (FENEMORE, 1980):.

Infestation category	**Damage category**	**Weighing factor**
1. Clean.	No visible- sign of infestation.	0
2. Slight.	One or two mines, which could be removed readily on peeling.	X_1
3. Moderate.	More than two mines present, and up to one-third of surface showing damage.	x_2
4. Severe.	More than one-third of the tuber surface showing damage.	x_3

The mean damage index of tuber (D.I.) for each experiment could be determined by the formula given by FENEMORE (1980).

The maximum possible index is, thus, 30 tunnels/tuber if all tubers fall into the severe category according to FENEMORE

$$D.L = \frac{(\text{no. slight}X_1) + (\text{no. moderate } X_2) + (\text{no. severe } X_3)}{\text{Total number of tubers}} \times 10$$

(1980). Each test was repeated five times.

Another experiment was carried out to compare the effectiveness of lower oil volumes (0.0125 and 0.025 ml /500 ml air of the tested container) of the most effective oils from the aforementioned survey by the higher concentration (0.05ml oil / 500 ml air). The same aspects were determined during the experimental period.

All tests were replicated ten times under laboratory conditions 28 ±2 C°.

b - Treatment of eggs

Deposited eggs of PTM on potato tuber surface were counted by using magnifying lens. They were placed in the container (500 ml) continuously exposed to vapors of different volumes (0.0125, 0.025 and 0.05ml/500ml) of plant oils or commercial monoterpenes as mentioned before in treatment of adults. Control experiments were used without treatment. Each treatment was replicated five times in separate containers. The percentage of egg hatchability was determined. The treated eggs were examined under a magnifying lens and compared with untreated ones. If the exposed eggs failed to hatch but showed development beyond the stage when tested oils were applied, it was assumed that failure to hatch was due to inhibition of embryonic development rather than acute toxicity of the oils vapors according to Saxena and Sharma (1972).

B - Dusting potato tubers

Two experiments were carried out: Different concentrations (0.25, 0.5 and 1%) of Bergamote (*Mewtfza citrata),* lemongrass *(C. citratus*\- Nutmag *(Myristica fragrans)* and ά-Ionone were prepared and mixed with a carrier material (Talcum powder). They were left for ten minutes at room temperature to dry, and used as dust.

In first experiment, 2-3 potato tubers (about 200gm) were dusted with one of the prepared dusting materials. The dusted tubers were introduced into containers (20 x 20 x 10 cm) where five pairs of virgin adults were confined. The following biological aspects were studied: (1) Average number of deposited eggs. (2) Percentage of emerged offspring, (3) Duration of life span (egg to adult (4) Damage index of potato tubers.

In the second experiment, 50 neonate larvae were introduced in a test chamber (20x20x10 cm) containing 1-2 dusted potato tubers (about 100 g). The percentage of penetrating larvae and percentage of pupation were determined. Each test was replicated five times.

C . Spraying gunny sacks

To protect sound potatoes kept at gunny sacks from PTM infestation,

gunnysacks (14 x 22 cm) were treated as follows:

Different concentrations (0.25, 0.5 and 1%) of the tested plant oils alone or combined were mixed with water. Two drops of Tween 80% were added as emulsifier to ensure homogenous water emulsion. The gunny sacks were sprayed and left for dryness before potato tubers were kept in them. Each treatment contained 2-3 (about 200gm) potato tubers , previously exposed to artificial infestation by three pairs of virgin male and female moths. The following aspects were studied: (1) Number of progeny (full grown larvae, pupae and emerged adults) after 25 days of infestation; (2) Damage index of potato tubers; (3) Persistence index of the residual materials.

Residual effectiveness experiments were conducted to estimate the biological persistence of the tested natural plant oils, commercial monoterpenes when being applied at highly effective concentration (or the maximum possible concentration on potato tubers (dusting), on gunnysacks (spraying) or in the storage chamber (fumigation) according to MANSOUR *et al.* (1997). The residual effectiveness was bioassayed using various stages of PTM according to the method of protection; thus: In case of treatment of PTM adult stage by natural plant oils and commercial monoterpenes (Concentration 0.05 ml/500 ml air exposure in glass jar) for fumigation or 1% conc., for spraying and dusting application) were used. Dusted potato tubers, sprayed sacks and fumigated containers were kept at room temperature, alongside with untreated samples. At different time intervals, samples of dusted tubers, treated gunnysacks or potato kept at fumigated container were taken for residual effectiveness assessment by subjecting them to neonate larvae or adult stage of PTM. According to the methods of protection, the following parameters were recorded: (1) Mean damage index, for all methods of application mentioned before; (2) Reduction percent in life span of adult stage exposed to vapors of plant oils or commercial monoterpenes (Reduction = %x-y, where x= length of life span of untreated moth, y= length of life span of treated moth); (3) Reduction percent of progeny found in the tubers kept in treated gunny sacks; (4) Percentage of larval mortality; when neonate larvae fed directly on the treated tubers. Tests for residual effectiveness were continued till reaching a degree of protection to > 5 0 %value.

Statistical analysis. All data were statistically subjected to analysis of variance (ANOVA) through "SPSS" Computer program.

Results and Discussion

1. Effect of vapors of natural plant oils and commercial monoterpenes.

a - On adult life spane and fertility.

Results on the effects of vapors of ten natural plant oils and three commercial monoterpenes (at 0.05 m oil/500 ml air) on adult life (virgin male and female moths) and the produced offspring are given in Table 1. It was clear that, there was variable reductions in the life span of both virgin male and female moths continuously exposed to vapors of tested plant oils comparable to control moths. Thus, the average life span of female PTM was highly reduced when exposed to fumes of *C. citratus, M. fragrans* (Nutmag and Mace oil) and *M. citrate* to reach 0.8, 1.2, 3.3 and 2.8, while that of male moths was reduced to reach 1.7, 1.3 0.5, 2.5, 0.9 and 3.8 days, respectively; compared to untreated female and male moths (control); being 10.8 and 10.0 days, respectively at (P>0.01).

The remaining natural plant oils and commercial monoterpenes (except ά-ionone) show low significant effect on the life span of exposed moths comparable to the unexposed ones (P > 0.05). On the other hand, most of the tested oils were highly effective on the PTM fertility. Thus, the emerged adult offspring of female moths continuously exposed to fumes of *Cymbopogon, Myristica (nutmag* oil), *Mentha* and ά-Ionone were reduced to reach 0.5, 0.5, 1.5and 2.3 offspring/female, respectively. The difference from that of the untreated female moths 34.3 offspring/female was statistically extremely significant (P < 0.01).

The other tested oils caused a moderate reducing effect on the fertility of adults exposed to fumes of included *Cinnamonium zeylanicum, M. fragrans* (Mace oil) and *P. graveolens* as their average numbers of emerged offspring were 11.8, 11.8and 24.4 offspring/female, respectively. The remaining tested oils showed insignificant effects on the fertility of tested moths as compared with the control (P > 0.05). Although, only few of the tested oils (at 0.05 ml / 500 ml air) succeeded to reduce the life span of exposed adults, many of them affected their fertility. Therefore, the most effective components were selected and tested at lower vapor emanating from oil concentrations (0.025 ml, and 0.0125 ml/500ml air).

Table 1. Effect of natural plant oils vapors and commercial monoterpenes at 0.05 m1/500m1 on the life span and emerged offspring of continuously exposed PTM.

Tested oils (at 0.05ml/500ml)	**Life span (days)**		**No. emerged adult offspring/female mean± S.D.**
	Female mean ±S.D.	**Male mean ±S.D.**	
Cinnamonium Zeylanicum	11.5±0.6^{a}	11.3±2.9^{a}	11.8±3.7^{c**}
Cymbopogon citratus.	0.8±0.2^{b} **	1.7±0.5^{b**}	0.5±0.2^{d**}
Eucalyptus ro.stratits.	13.8±1.8^{a}	6.5±4.9^{a}	27.0±5.6^{a}
Janiperus virginiana	8.3±0.6^{a}	9.5±1.1^{a}	24.3±2.8^{b*}
Mentha citrata	2.8±0.6^{b} **	3.8±1.1^{b**}	1.5±0.5^{d**}
Myristica fragrans (Mace)	3.3±1.1^{b} **	2.5±0.9^{b**}	11.8±1.9^{c**}
Myristica fragrans (Nutmag)	1.2±0.7^{b} **	1.3±0.5^{b} **	0.5±0.2^{d**}
Pelargonium graveolens	8.3±1.4^{a}	11±1.6^{a}	24.4±3.4^{b*}
Syzygium aromaticum	9.3±0.6^{a}	6.5±0.4^{a}	26.0±5.9^{a}
Zingiber officinale	11.3±0.7^{a}	11.5±1.3^{a}	15.5±4.3^{c**}
B-Cmmercial monoterpenes.			
Geraniol	9.3±1.5^{a}	8.8±2.1^{a}	27.3±3.03^{a}
Isoeugenol	10.3±0.8^{a}	10.8±0.5^{a}	34.3±3.8^{a}
α - lonone	4.6±0.9^{b} *	5.8±1.5^{a}	2.3±1.4^{d} **
Control	10.8±1.1^{a}	10.3±1.2^{a}	34.3±2.8^{a}

$L.S.D_{0.05}$ =4.6 $L.S.D_{0.05}$ = 9.9

$L.S.D_{0.01}$ = 6.09 $L.S.D_{0.01}$ = 13.6

Means with the same letters in vertical columns are not significantly different (P >0.05).

** significant at (p>0.05 , ** highly significant at (p> 0.01).*

Our results in agreement with recorded by Kordail et al, (2005) that the essential oils such as lemon grass (*C. winteriana*), *E. globulus*, rosmary (*R. officinalis*), vetiver (*Vetivera zizanoides*), Clove (*Eugenia caryophyllus*) and thyme (*Thumus vulgaris*) are known for their pest control properties. While peppermint (*M. piperita*) repels ants, flies, lice and moths, pennyroyal (*M. pulegium*) ward off fleas, ant, lice, mosquitoes, ticks and moths. Spearmint (*M. spicata*) and basil (*O. basilicum*) are also effective in warding off flies.

Results given in Table 2 indicate, that the effectiveness was concentration dependence; i.e. higher concentration caused higher reduction in the life span and fertility of exposed adult moths. Thus, in case of treatment by *C.citratus* oil, it was found that the average life span of exposed female and male moths decreased from 5.8 and 5.8 days to 3.6and 4.5 days, till it reached 0.8 and 1.7 days, respectively, at concentrations 0.0125, 0.025 and 0.05 ml / 500 ml air, respectively. Treatment of PTM by *M.fragrans* (nutmag) oil, also caused a reduction in the life span of exposed female & male moths from 6.3 and 4.8 days at 0.0125 ml to 4.0and 3.5 days at 0.025 ml, till it reached 1.3and 1.6 days at 0.05 ml / 500 ml air for female and male moths, respectively. An almost similar trend was reached, in case *of M. citrata* oil and ά-Ionone (commercial monoterpene). On the other hand, the average number of emerged offspring was severely affected by the different concentrations of the tested oils comparable to the control. However, *Cymbopogon* and *Myristica* oils did not elicit potent variation ($P > 0.05$) between 0.0125 and 0.025 ml / 500 ml air; but the effectiveness became highly significant ($P < 0.01$) when comparing these two concentrations with the higher concentration (0.05 ml / 500 ml air). Thus, the average emerged offspring was 11.5, 10.5 and 0.5 in case of *Cymbopogon* oil, and 15.8, 12.5 and 0.5 in case of *Myristica* oil at concentrations 0.0125, 0.025 and 0.05 ml/ 500 ml air, respectively. Treatments by ά-Ionone (terpene) and *Mentha* (oil) exhibited significant potent difference ($P < 0.05$) between 0.0125 and 0.025 ml; but highly significant difference ($P < 0.01$) with higher concentration (0.05 ml / 500 ml air). Thus, the average emerged offspring was 29.0, 21.8and 2.3 in case of a-Ionone, and 14.7, 9.3 and 1.5 in case *oil M. citrata* oil at concentrations 0.0125, 0.025 and 0.05 ml / 500 ml air, respectively. Based on the present findings, it can be concluded that *C. citratus, M. fragrans* (nutmag), *M. citrata* and a-Ionone at a high concentration (0.05 ml / 500 ml air) can successfully reduce potato infestation by the potato tuber moth, through shortening the life span of adults and reducing their fertility. As the majority of exposed females died before laying eggs; and failure of others to lay their full load of eggs. In this respect, Sharaby (1988) showed that the fecundity and fertility of the potato tuber moth were significantly reduced when moths of either sex were exposed to vapors of orange peel oil emanating from 160 uLoil in 250 ml glass jars. Oviposition and egg hatching were totally inhibited when female moths were exposed to 220 uLof the oil. EL-NAHAL *et al* (1989) found that vapors of the essential oils of *Acorus calamus* (L.) rhizomes had toxic effect against adults of several stored product insect species; and also confirmed that the period of exposure appeared to be the most important factor affecting the efficiency of these vapors rather than the dosage. Stamopoulos (1991) tested four essential oils (geranium, cypress, eucalyptus and bitter almond) in their vapor form against *Acanthoscelides obtecutus* (Say). He found that eucalyptus vapor strongly reduced fecundity, decreased egg hatchability and increased neonate larval mortality. schmidt *et al* (1991) showed that the numbers of produced offspring of *Sitophilus granarius* (L.), *S. oryzae* (L) and *Callosobruchus chinensis* (L.) emerging from food, on which adults were placed during and after treatment with *A, calamus* oil vapours were lower than in the respective control. The author correlated that to the increase of exposure time rather than to

increase in dose. It has also been shown that aromatic plants (as *Labiatae sp.)* were the most active in the protection of *Phaseolus vulgaris* (L.) from *A. obtecutus* infestation. These plants are rich in essential oils, which show vapor toxicity toward adults, and inhibit reproduction by ovicidal and larvicidal effects (REGNAULT-ROGER & HAMRAOUI, 1993, 1994). REGNAULT-ROGER & HAMRAOUI (1995) showed that most of the volatile monoterpenes as carvacol, Linalool eugenol and others exhibited the highest fumigant toxic effect on the adult stage of *A. obtecutus,* beside inhibiting reproduction. In addition, they found that monoterpenes cumulatively affected the survival of the beetle but no compounds acted with the same intensity at each developmental stage. However, as far as the writer is aware, the mode of action of the tested oils is not exactly known, and further studies have to be carried out especially to clarify how they are involved in the physiology of reproduction. The rapid action against some pests is indicative of a neurotoxic mode of action, and there is evidence for interference with the neuromodulator octopamine (KOSTYKOVSKY *et al.,* 2002)

Table 2. Effect of different concentrations of vapors of natural plant oils and commercial monoterpenes on the life span and emerged offspring of PTM.

Tested oils	Conc. Ml/500ml	Life span (days)		No. emerged adult offspring/female mean + S.D.	% of egg hatching
		Female mea ±S.D.	Male mean±S.D.		
A- Natrual oils.					
Cymbopogon citratus	0.0125	$5.8\pm0.6^{ca*}$	$5.8\pm0.6^{ca*}$	$11.5\pm2.5^{ca*}$	75.2
	0.025	$3.6\pm0.6^{db*}$	$4.5\pm1.04^{ca*}$	$10.5\pm3.1^{ca*}$	67.9
	0.05	$0.8\pm0.2^{dc*}$	$1.7\pm0.5^{dc*}$	$0.5\pm0.2^{dc*}$	41.3
Mentha citrata	0.0125	$6.8\pm1.1^{ca*}$	$6.0\pm0.5^{ca*}$	$14.7\pm2.4^{ca*}$	85.7
	0.025	$5.8\pm0.6^{ca*}$	$4.5\pm0.6^{ca*}$	$9.3\pm1.1^{cb*}$	76.8
	0.05	$2.8\pm0.6^{cb*}$	$3.8\pm1.1^{cb*}$	$1.5\pm0.5^{dc*}$	62.2
Myristica fragrans (Nutmag)	0.0125	$6.3\pm1.5^{ca**}$	$4.8\pm0.8^{ca**}$	$15.8\pm2.6^{ca**}$	73.6
	0.025	$4.0\pm0.5^{ca**}$	$3.5\pm0.6^{ca**}$	$12.5\pm2.9^{ca**}$	70.96
	0.05	$1.3\pm0.5^{cb**}$	$1.6\pm0.8^{dc**}$	$0.5\pm0.2^{dc**}$	43.3
B-Cmmercial monoterpenes.					
α- lonone	0.0125	$7.8\pm1.03^{ba**}$	$8.8\pm1.1^{ca***}$	$29.0\pm1.8^{aa**}$	79.8
	0.025	$7.3\pm1.03^{ca**}$	$7.8\pm0.6^{ca**}$	$21.8\pm1.3^{cb**}$	77.5
	0.05	$6.4\pm0.9^{ca**}$	$5.8\pm1.5^{cb**}$	$2.3\pm1.4^{dc**}$	60.2
Control	---	10.8 ± 1.1^{a}	10.3 ± 1.9^{a}	34.3 ± 2.8^{a}	96.5

$L.S.D_{0.05}$ = 2.43 *$L.S.D_{0.05}$ = 5.8*

$L.S.D_{0.01}$ = 3.22 *$L.S.D_{0.01}$ = 8.1*

Means with the same letters in vertical columns are not significantly different (P>0.05).

** significant atp> 0.05.,** highly significant at p>0.01.*

b - On egg hatchability

The effectiveness of vapors of different concentrations of four tested oils on egg hatchability of the potato tuber moth is given in Table 2. Results obtained indicated that the higher the concentration of the oil, the lower the percentage of egg hatchability. Thus, all tested oils could reduce the hatchability of eggs of PTM and the percentage of egg hatchability was oil dose dependent. Moreover, vapors of *Cymbopogon* and *Myristica* oils appeared to be the most effective as they caused the highest reduction in the percentage of egg hatchability at all tested concentrations. The obtained results concerning the role of

vapors of volatile oils in reducing egg hatchability are in agreement with SCHMIDT et al. (1991) who recorded that egg hatchability of *Callosobrusus chinensis* (L.) was reduced after 96hr. exposure to vapors of *Acorus calamus* (L.). They attributed that to the toxicity of the vapors to eggs. *Citronella* (*C. nardus*) essential oil has been used as an insect repellent and an animal repellent. Combining few drops each of citronella, lemon (*Citrus limon)*, rose (*Rosa damascene*), lavender and basil essential oils with one liter of distilled water is effective to ward off indoor insect pests. The larvicidal activity of, citronella oil has been attributed to its major monoterpenic constituent citronellal (ZARIDAH *et al.,* 3003). In addition, PATHAK & KRISHNA (1992, 1993) observed high mortality following exposure of eggs of *Corcyra cephalonica* (Stainton) and eggs of *Earias vitella* (F.) to vapors of eucalyptus and clove oils, respectively. GURUSUBRAMANIAN & KRISHNA (1996) found that severe reduction in egg hatchability occurred in *Earias vitalla* (Fabricius) and *Dysderus koenigii* (F.) when their eggs were exposed to the vapour of *Alluim sativum.* They attributed that to some chemical ingredients present in the volatiles of *A. sativum* (bulbs) which probably diffused into eggs and affected the vital physiological and biochemical processes associated with embryonic development. The embryonic development in these eggs was not relatively complete; and the egg color did not change from crystal – transparent to dark color as in the control eggs.

c - The damage assessment of potato tubers exposed to vapors of natural plant oils and commercial monoterpenes.

Data on protection of stored potato tubers by using vapors of ten oils and three commercial monoterpenes (at 0.05 ml/500 ml air) are given in Table 3. By screening a large number of natural plant oils and commercial monoterpenes, it was clear that few numbers of the tested materials had the ability to protect potato tubers from PTM infestation. The most effective oils which gave the lowest tuber damage index, could be arranged in an ascending order as follows: *M. fragrans* (nutmag) < *C. citratus* < *M. citrata* < a-Ionone; showing 2.4 ± 0.8, 2.5 ± 1.5, 4.1 ± 1.6 and 6.6 ± 1.4 tunnels/tuber, respectively, compared to 30.0 ± 0.0 tunnels / tuber in the control. The difference is highly significant ($P < 0.01$).

Table 3. Damage index of potato tubers treated with vapors of natural plant oils and commercial monoterpenes.

Tested oils (at 0.05 ml / 500 ml)	Tuber damage index (tunnels / tuber) mean ± S. D.
A-Natural plant oils :	
Cinnamonium zeylanicum	27.5± 0.9 ^{a}
Cymbopogon citratus	2.5± 1.5 $^{c **}$
Eucalyptus rostratus	25.8± 1.6 ^{a}
Janiperus virginiana	26.6± 1.4 ^{a}
Mentha citrata	4.1± 1.6 $^{c **}$
Myristica ragrans (Mace oil)	21.3± 3.3 $^{b **}$
Myristica fragrans (Nutmag oil)	2.4± 0.8 $^{c **}$
Pelargonium graveolens	23.3± 1.3 ^{b**}
Syzygium aromaticum	24.1± 1.6 $^{b **}$
Zingiber officinale	22.5± 1.2 ^{b**}
B- Commercial monoterpenes:	
Geraniol	23.3± 1.3 $^{b **}$
Isoeugenol	28.3± 1.02^{a}
x-Ionone	6.6± 1.4 $^{c **}$
Control	30.0± 0.0 ^{a}

$L.S.D_{0.05} = 5.04$ $L.S.D_{0.01} = 6..9$

Means with the same letters have no significant difference ($P > 0.05$).

* significant at $p>0.05$., ** *highly significant at p>0.01.*

On the other hand, a moderate damage was recorded in tubers exposed to vapours of Geraniol (monoterpene), *P. graveolens, M. fragrans* (Mace oil), and *Z. officinale* (natural oil), where the average damage indices were 23.3, 23.3, 21.3and 22.5 tunnels /tuber, respectively. The difference from the control is statistically significant ($P < 0.05$). The remaining oils and monoterpenes did not record any significant difference ($P > 0.05$) from the control.

Damage index of potato tuber treated with natural oils and commercial monoterpene shows the role of lower concentrations of the most effective of the aforementioned oils (0.025 ml and 0.0125 ml / 500 ml air) in protecting potato tubers against PTM damage are given in Table 4. The obtained data showed highly significant difference ($P < 0.01$) in tuber damage indices among different concentrations of each oil. The effect was dose dependent, as higher doses showed the least tuber damage index; indicating their efficacy in tuber protection. In case of treatment by using *M. citrata* oil, the mean damage index of tubers decreased from 25.8 ± 1.6 tunnels/ tuber at 0.0125ml/ 500ml., till it reached 4.1 ± 1.6 tunnels / tuber 0.05 ml/500 ml concentrations. An almost similar trend was reached in case of *C.citratus* and *M. fragrans* (nutmag oil) which appeared the most effective, while a-Ionone (monoterpene) appeared the least effective, particularly at lower concentrations (0.0125 and 0.025 ml / 500 ml). Similarly, BEKELE *et al.* (1997) found that exposure of adults of *Sitotroga cerealella* (Oliv.) to higher dosages of ground leaves and essential oil extract of *Ocimum kenyense* (Ayobangira) induced 100% mortality within 24hr., and reduced feeding, indicating high protecting potency against the insect damage. SHAAYA *et al.* (1997) used vapors of a large number of essential oils extracted from various spice and herb plants against several major stored product insects. They found that *Labiatae sp.* oil (ZP_5j) at higher concentrations (1.5 - 4.5$\mu L/ l$ air) caused 90% mortality of all insects in space tests after 24 hr. of exposure. They suggested that most of plant oils can play an important role in stored grain protection and reduce the need for and risks associated with the use of insecticides. Essential oil constituents are primarily lipophilic compounds that act as toxins, feeding detterents and oviposition deterrents to a wide variety if insect pests, it was also effective as a fumigant (KOUL *et al.*, 2008).

B -Effect of dusting potato tubers with natural plant materials on certain biological aspects of PTM.

Potato tubers were treated by different concentrations of the tested materials (natural plant oils and commercial monoterpene) using the dusting technique materials. Treated tubers were exposed to the potato tuber moth for eggs laying. All the biological aspects of the pest were followed up. Results on the persistence index of dusting potato tubers with plant oil and monoterpene (at 1% cone, with talcum powder) against larvae of PTM are presented in Table 5. It was clear that, all tested materials caused initially high larval mortality and low damage index. They, however, showed variable degardative periods. Thus, treatment of potato tubers with *Cymbopogon* and *Mentha* oils (1% in talcum powder) initiated (after one day) high percentages of larval mortality; being 88.8 and 87.0%, respectively, and low damage index; being 2.4 tunnels / tuber for each of them. After 20 days of storage in cas *of Cymbopogon* and 15 days of storage in case of *Mentha,* the final larval mortality decreased to reach 23.5 and 28.05%, with damage indices of 19.2and 22.4 tunnels/ tuber, respectively. This led to 35.1 and 24.3% protection, with 5.29 and 4.84 persistence index, respectively. On the other hand, treatment of potato tubers with *Myristica* and α-Ionone caused initially 82.6 and 83% larval mortality; showing damage indices 5.2 and 4.4 1.1 tunnels / tuber, respectively. After 10 days in case *of Myristica* and 15 days in case of ά- lonone, the final effect was reached, where the larval mortality was 39.0 and 24.7%, respectively, with a damage index of 18.4 tunnels/ tuber and 37.8% protection for each of them. This led to 4.72 and 4.46 persistence index, respectively.

Table 4. Damage index of potato tubers treated with different concentrations of natural plant oils and a commercial monoterpene

Tested oils	Conc. ml/500 ml	Tuber damage index (tunnels / tuber) mean-± S. D.
A- Natural plant oils :		
Cymbopogon citratus	0.0125	17.5 ± 2.5 bc **
	0.025	5.0 ± 2.1 dc **
	0.05	3.3 ± 2.3 d c**
Mentha citrata	0.0125	25.8 ± 1. 6 b *
	0.025	12.5 ± 3.8 cb **
	0.05	4.1 ± 1.6 dc**
Myristica fragrans (nutmag oil)	0.0125	13.3 ± 2.7 cb **
	0.025	7.5 + 2.1 cb**
	0.05	2.7 ± 0.3 dc**
B- Commercial monoterpene:		
a-Ionone	0.0125	23.3 ± 1.3 ba *
	0.025	19.9 ± 2.4 da*
	0.05	4.1 ± 1.6 dc*
Control	---	30.0 ± 0.0 a

$L.S.D_{0.05}$ 5.04 $L.S.D_{0.01}$ = 6.9

Means with the same letters in vertical columns for each oil concentration have no significant difference (P > 0.05). * significant at p>0.05., ** highly significant at p> 0.01.

C - Persistence index of plant oils sprayed on gunnysacks.

The results obtained in Table(6)shows that, spraying natural plant oils separately or mixed together to gunny sacks resulted in various levels of reduction of PTM progeny and damage indices, throughout the storage period. Thus, the highest initial percentages of reduction of PTM progeny were 95.7, 95.7 and 94.3% after one day of storage in gunny sacks sprayed with 1% *Myristica, Cymbopogon* + *Myristica* and *Cymbopogon,* respectively. These gradually decreased to reach 47.1, 49.3 and 48.4% after 20 days of storage with respective damage indices of 18.4, 18.4 and 19.9 tunnels/ tuber, respectively. So their persistence indices were 9.84, 10.30 and 7.14, respectively. Treatment of the gunny sacks with *Mentha* + *Cymbopogon* and *Mentha* + *Myristica,* on the other hand, exhibited a moderate initial reduction in PTM progeny after one day of storage, showing 89.95 and 78%, respectively. These gradually decreased till reached 48.9 and 40.7% after 15 days of spraying gunny sacks; with damage indices of 18.4 and 19.2 tunnels / tuber, respectively. This led to 8.15 and 7.83 persistence index, respectively. *Mentha* oil or the three oils when mixed together caused relatively less reduction in the percentage of PTM progeny after one day of spraying the gunny sacks; being 51.2 and 60.8%, respectively. Their potency highly decreased after 10 days of storage causing *32,6%* progeny reduction in case of *Mentha;* and 42.7% in case of mixed oils after 5 days of spraying gunny sacks; so their persistence indices were 7.14 and 3.43, respectively. It was noticed that, the best protection occurred after one day of storage for all tested oils and then gradually decreased till it reached a lower level after variable periods according to theoil used. Accordingly, their persistence indices varied, and was highest in case of oils of *Myristica, Cymbopogon* and their mixture together; as they remained potent for more than 20 days after spraying. These observations agree with Sharma *et al* (1983) who found that treatments of the Jute or tarpaulin cover

spread over stored potatoes with 10% neemrich oil extract provided prophylactic measure against the potato tuber moth. In this respect, Abdalla and Matter (1994) also recorded that spraying covers of potato heaps with lemongrass or neem extract significantly reduced moth infestation up to 2 and 3 weeks for storing, respectively; while mixing both extracts did not significantly increase their action. From the present results, it could be concluded that most plant oils could exhibit good protection for long periods when used as vapours against PTM, sprayed in gunny sacks or used as powder dusted on potato tubers. However, dried plant powders gave much longer protection of the potato tubers from PTM infestation. Raman *et al* (1987) mentioned that *E.globulus, Lantana camara* and *Minthostachys* sp., both in the dried shredded and powder form, were effective in controlling PTM damage in potatoes for 4 months; while treatment of potato tubers with vegetable oils or neem extract did not reduce PTM damage or tuber rot for long storage period. Similarly, Khashyap *et al* (1992) tested the efficacy of *Cannabis sativa* dry leaves powder in controlling PTM in stored potatoes in India. They found that 2cm. thick layer of *C. sativa* protected potatoes for up to 120 days. Clove oil and their role in protecting stored products from target insects were studied. For example, Sighamony *et al* (1986) studied the efficiency of Clove, Ceder wood and Karanja oils at doses of 25 -100 ppm against *Sitophilus oryzae* and *Rhyzopertha dominica.* Their results indicated that all tested oils had the ability to protect wheat grain from insect infestation up to 60 and 30 days,respectively, after exposure. The persistence of toxicity ranked Clove oil > Karanja oil > Ceder wood oil against *S. Oryzae;* while in case of *R. dominica,* both toxicity and persistence were lower and ranked Karanja oil > Ceder wood oil > Clove oil. Bhaduri *et al* (1990) found that treatment of bean seeds with *C. nardus* and *C. citratus* oils exhibited protection up to 90 days from *Callosobruchns maculatus* (F.) infestation.

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Table 5. Persistence index of dusting potato tubers with natural plant oils and a commercial monoterpene against PTM larvae

Duration of storage period / Tested oils (1%)	One day		5 days		10 days		15 days		20 days		Residual efficiency (F/I)	*Persistence* index (F/I X days)
	Larval mortality (%)	Average D. L	Larval mortality (%)	Average D. I.	Larval mortality (%)	Average D. I.	Larval mortality (%)	Average D. I.	Larval mortality (%)	Average D. I.		
A. Natural plant oils												
Mentha citrate	I 87	2.4 ± 1.2 (91.5)	86.5	6.2±2.03 (79.1)	51.3	14.8±1.5 (50)	F 28.05	22.4±1.5 (24.3)	16.7	27.6±0.6 (6.8)	0.322	4.84
Cymbopogon citratus	I 88.8	2.4±1.2 (91.5)	73.8	4.0±0.9 (86.5)	60.5	10.4±1.4 (64.9)	41.8	11.6±1.8 (60.8)	F 23.5	19.2±0.7 (35.1)	0.265	5.29
Myristica fragrans	I 82.6	5.2±1. 4 (81.7)	64.3	12±2.2 (59.5)	F 39.0	18.4±2.1 (37.8)	21.6	17.2±1.5 (41.9)	19.5	26±1.1 (12.2)	0.472	4.72
B- Commercial monoterpe e												
ά- Ionone	I 83	4.4±1.1 (84.5)	77.6	7.8±0.7 (73.6)	50	11.6±1.2 (60. 8)	F 24.7	18.4±2.1 (37.8)	11.8	26±1.I (12.2)	0.298	4.46
Control (Talcum powder)	I 1.0	28.4±.0.7	11.0	29.6±0.5	12.6	29.6±0.5	10.8 .	29.6±0.8 .	11.0	29.6±0.5	-	-

$L.S.D_{0.05}$ = 4.06 $L.S.D_{0.05}$ = 10.5

I= initial effect; F = final effect, D.I. = damage index

I= initial effect; F = final effect, D.I. = damage index

- The values between brackets indicate % of protection (i.e, % of reduction in D.I.).
- Deviations in the table indicate the standard deviations of means.

Table 6. Persistence index of tested natural plant oils sprayed on gunny sacks against PTM infestation.

Duration of storage	One day		5 days		10 days		15 days		20 days		Residual efficiency (F/I)	Persistence index (F/I X days)
Tested oils (1%)	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.	Average Emerged progeny	Average D. I.		
Cympopogo n Citratus	2.4 ± 1.6 $_i$ (94.3)	0.5t0.5 (98.3)	9.8±2.4 (75.3)	1.2±0.8 (96)	13.4±2.7 (71.6)	4.8±0.8 (84)	14.6±2.2 (68.4)	11.6±1.8 (61.3)	22.8±2.2 F (48.4)	19.9±2.4 c33.7)	0.357	7.14
Mentha Citrata	20.4t 1.8	12.8±1.9 (5 7 . 3)	22±2. I (44.4)	14.4t1.7 (52)	31.4±3.03 F (32.6)	7.2±2.2 (42.7)	38.0±2.02 07.7)	24.8±1.5 (18.7)	38±2.1 (14.02)	24.8±1.5 (18.7)	0.637 0.637	6.37
Myristica fragrans	1.8±1.2 i(95.7)	0.5±0.5 (98.3)	8.2±1.9 (79.3)	4.4±1.1 (85.3)	11.8±2.8 (74.7)	8.4±2.0 (72)	18.2±2.7 (60.6)	11.6±1.8 (61.3)	23.4±3.1 F (47.1))	18.4±2.1 (38.0	0.492	9.84
Menthe + Myristica	9.2±1.9 i (78)	5.6±2.2 (81.3)	15.4±1.6 (61.1)	6.2±2.5 (79.3)	20.2±2.1 (56.7)	14.8±1.5 (50.7)	27.4±2.01 F (40.7)	19.2±0.7 (36.0) ..-	30.4±2.9 (31.2)	21 2±1.6 (29.3)	0.521	7.83
Menthe + Cyinbopogon	4.2±2.2 i (89.95)	1.21-0.8 (98)	17.4±2.1 (56.06)	6.2±2.1 (79.3)	17.9±3.7 (61.6)	6.2±1.9 (79.3)	23.6±2.5 *F* (48.9)	18.4±1.9 (38.7)	28.6±2.7 (35.3)	22.4±1.8 (25.3)	0.544	8.15
Cymbopogon + Myristica	1.8±1.2 1(95.1)st	0.5±0.5 *(98.3)*	7.4±1.97 (81.3)	4.0±0.9 (86.7)	12.8±2.03 (72.5)	6.2±2.1 (79.3)	18±2.4 (61.04)	11.6±1.8 (61.3)	22.4±2.1 F (49.3)	18 4±1.9 (38.7)	0.515	10.30
Cymbopogon + Myristica + Mentha	16.4t3.8 (60.8)	7.8+0.7 (74)	23.1±1.9 F (41.7)	19±0.8 (36.6)	27.6±2.5 (40.8)	19.2±0.7 (36)	38±0.9 (17.7)	22.4+1.5 (49.3)	40.2±1.7 (9.05)	26 0±1.1 (13.3)	0.686	3.43
Control	41.8±2.3	30±0.0	39.6±3.9	30±0.0	46.6±2.9	30-0.0	46.2±3.1	30±0.0	44.2±3.7	30±0.0	-	-

1= initial effect; F = fmal effect D.I. = damage index

- For each storage period, the values between brackets in the left columns represent the % of reduction in PTM progeny; while those in the right columns the % of protection (i. e., % of reduction in D. I.)
- Deviations in the table indicate the standard deviations of means.

L.S.D 0.05 = 4.9 L.S.D 0.01= 6.7

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Effects of Drought on Plant Species Diversity and Productivity in the Oak Forests of Western Iran

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Abstract. A severe drought in 2008 extensively damaged a variety of economic, social, agricultural and natural resources in Iran. This study investigated the effects of the 2008 drought on plant species composition, diversity and productivity in Western Iran. To this end, plant species diversity in the drought year (2008) was compared to pre-drought (2007) and post-drought (2009) diversity. The Shannon-Wiener diversity index and Margalef richness index had significant differences between years, decreasing significantly during drought and significantly increasing post-drought. In contrast, the Smith-Wilson evenness index did not significantly differ between years. Plant dry weight was significantly reduced by drought and increased significantly post-drought. The percent cover of sixteen species was significantly reduced in the drought year. Furthermore, nine species disappeared during drought, but reappeared after precipitation. The most sensitive species to drought were *Psathyrostachys fragili, Carex sp., Falcaria falcarioide, Festuca ovina* and *Scariola orientalis*. Five species *(Cardaria draba, Echium amoenum, Polygonatum orientale, Medicago noeana* and *Cirsium vulgare)* not present before and during drought appeared the year after drought ended. Some of the effects of drought may be reduced by improved land management planning and water conservation to better provide for the water needs of Iran and other drought-prone countries.

Keywords: Drought, Precipitation, Plant Diversity, Productivity, Western Iran.

Introduction

Water availability is the primary limitation to plant productivity in many terrestrial biomes and it is an ecosystem driver that will be strongly affected in many areas of the world by ongoing and future climate change (HEISLER-WHITE *et al*., 2008). Recent climate models predict that the 21st century will be characterized by increasing temperatures, changing precipitation patterns and more frequent extreme events such as heat waves and droughts (SCHAR *et al*., 2004) that will exacerbate land degradation and desertification (MEADOWS & HOFFMAN, 2003). Drought-related ecological degradation, including forest dieback, grassland desertification, wetland degradation, and Lake Desiccation have been widely reported, especially in semi-arid regions (YIN *et al*., 2012).

Ecological vulnerability to climate change depends on the ability of natural ecosystems to cope with stresses to biological systems (SCHROTER *et al*., 2005). One anticipated effect of climate change is expected to be loss of species (IPCC, 2001). Drought has major impacts on the composition, structure and function of vegetation (ALLENA *et al*., 2010). Drought can inhibit photosynthesis, cause mortality, create conditions for outbreaks of plant diseases and insect pests, and increase the

frequency and intensity of fire disturbance. Cumulatively, these factors can alter plant communities, causing extensive mortality potentially endangering survival of some plant populations and lowering total primary productivity of terrestrial ecosystems, accelerating the loss of ecosystems in fragile areas, and even endangering regional biodiversity (WANG *et al.*, 2010). There are three ways which plants may respond to a climatic change: persistence in the modified climate, migration to more suitable climates, or extinction (THEURILLAT & GUISAN, 2001). This study documents the response of an arid ecosystem in western Iran to a severe drought.

Climatic variability is a prominent feature of most ecosystems (HENDERSON-SELLERS & ROBINSON, 1991). Semiarid regions seem to be susceptible to drought (ALLEN & BRESHEARS, 1998). According to the United Nations (provide reference here), in the near future, 31 countries will experience serious water shortages, with Iran one of the most sensitive jurisdictions. UN research suggests that available water in 1990 of 2000 million m^3 will be reduced to 726-860 million m^3 in 2025 in Iran. Iran is the eighteenth largest country in the world, with an area of 1,648,195 km2. Iran has arid to semi-arid climate with low rainfall. Surface and subsurface water flow into Iran are very low. The main source of water is rainfall, which has an annual average of 250 mm. This amount is one-third of world and Asian rainfall. Furthermore, regionally, northern Iran receives annual average precipitation of 850 mm, while other parts of the country receive less than 50 mm.

Drought occurs somewhere in Iran almost every year. Despite the importance of drought and rainfall to Iran, its effects have only been studied in the agricultural and economic sectors, with no attention to natural resources, such as forests, that are important for forage and in preventing desertification. The aim of this study was to investigate the effects of drought on plant species composition, diversity and productivity in western Iranian forests. It is hoped that this research will lead to more attention on climate change and its effects on the vegetation of Iran.

Material and Methods

Study area

The study area (10000 ha) is located in the forests of Divandarreh, a city in Kurdistan province in western Iran (35°54′50″ N, 47°01′26″ E). Divandarreh is located in the Zagros Mountains of northern Kurdistan. The average altitude is 1850 m a.s.l. Annual temperature varies between -20 to +32 °C. The mean temperature of the warmest month of the year is 23.3 ° C and the mean temperature of the coldest month of the year is -7.3° C. The number of frost days is 135 per year. Average annual precipitation is 350 to 450 mm. Soils range in pH from 6.2 to 6.7. A severe drought occurred in 2008. Table 1 shows annual precipitation average in the 2007, 2008 and 2009 years for the nearest meteorology station (Fig. 1).

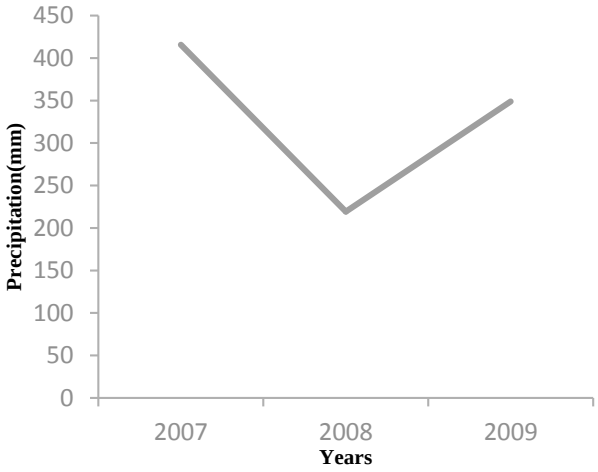

Fig.1. Total annual precipitation in the study area

Sample collection

Vegetation in 2007, 2008, and 2009 (pre-drought, drought, and post-drought years, respectively) was analyzed. The area sampled was determined using Whitaker's minimal area (POURBABAEI & POURRAHMATI, 2009), resulting in plants being analyzed on 64 m^2. Data collection was based on the Domin criterion (POURBABAEI & POURRAHMATI, 2009). In each year 30-64 m^2 sample plots were assessed. Plants were

segregated by species and dry weights measured after oven-drying for 72 hours at 75°C.

Data Analysis

To evaluate the effect of drought and precipitation on different aspects of plant biodiversity, we used three indices. Species diversity was assessed with the Shannon-Wiener index (MAGURRAN, 1988):

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

where P_i is the relative frequency of the i^{th} species.

Species richness was estimated according to the Margalef index (LUDWIG & REYNOLDS, 1988):

$$R_{Mn} = \frac{S}{\sqrt{N}}$$

where S is the total number of species and N is the total number of individuals.

Species evenness was calculated using the Smith-Wilson index (SMITH & WILSON, 1996):

$$E_{var} = \frac{2}{\pi \arctan\left\{\sum_{i=1}^{s}\left(\log_e(n_i) - \sum_{j=1}^{s}\log_e(n_j)/s\right)^2 \Big/ S\right\}}$$

where n_i is the number of individuals of the i^{th} species in a plot, n_j is number of individual of the j^{th} species, and S is the total number of species in U and UB areas.

All three indices were computed with software provided by KREBS (1989; Ecological Methodology for Windows, version 6.0).

Kolmogorov–Smirnov tests were used to test normality of all parameters. The significance of difference between means was analyzed using one-way ANOVA, followed by Duncan's mean separation test at the 95% level. Statistical analyses were performed using SPSS (version 18.0, SPSS Inc., Chicago, USA).

Results

In total, 42 species belonging to 15 families were present. The most common families were *Fabaceae* (8 species), *Asteraceae* (7), *Poaceae* (6), *Umbelliferae* (5), *Lamiaceae* (4), *Liliaceae* (2). *Cyperaceae, Euphorbiaceae, Poaceae, Chenopodiaceae, Plantaginaceae, Polygonaceae, Papaveraceae* and *Boraginaceae* families were each represented by only one species.

Greatest percentage ground cover by species in 2007 was, in order, *Astragalus* sp, *Gundelia tourneforti, Euphorbia aucheri, Phlomis kurdica, Ferula haussknechtii* and *Trifolium resupinatum.* In the drought year, greatest ground cover was found to be *Astragalus* sp., *Phlomis kurdica* and *Stachys lavandulifolia.* In the post-drought year, ground cover changed again, with *Astragalus* sp., *followed by Eryngium caucasicu, Echinops haussknechtii, Gundelia tournefortii, Euphorbia aucheri, Phlomis kurdica, Onobrychis andalanica* and *Trifolium resupinatum*.

Astragalus sp., *Echinops haussknechti, Gundelia tournefortii, Euphorbia aucheri, Phlomis kurdica, Ferula haussknechtii, Cynodon dactylon, Onobrychis andalanica, Bromus tectorum ,Thymus kotschyanus, Tragopogon buphthalmoides, Vicia koeieana, Rheum ribes, Kelussia odoratissima, Allium hitifolium* and *Glaucium contortuplicatum* had significant decrease in production at the end of the drought year. *Astragalus* sp., *Eryngium caucasicum, Echinops haussknechtii, Euphorbia aucheri, Phlomis kurdica., Ferula haussknechtii, Cynodon dactylon, Onobrychis andalanica, Bromus tectorum, Thymus kotschyanus, Tragopogon buphthalmoides, Vicia koeieana, Rheum Ribes, Glaucium contortuplicatum, Allium hitifolium* and *Kelussia odoratissima* species had significant increase in precipitation year. *Heteranthelium piliferum, Dactylis glomerata, Stachys lavandulifolia, Cicer anatolicum, Agropyrum kosaninii* and *Achillea kellalensis* species had no significance difference between three years.

Anthemis persica, Poa pratensis, Lotus gebelia, Grammosciadium platycarpum, Mentha longifolia, Glycyrrhiza glabera, Plantago atrata, Rapistrum rugosum and *Trifolium resupinatum* species were not present in the drought year but were found both before and after the drought, in 2007 and 2009. *Psathyrostachys fragilis, Carex* sp., *Falcaria falcarioides, Festuca ovina* and

Scariola orientalis species were only present in 2007 and did not reappear in the year immediately following the drought. *Cardaria draba, Echium amoenum, Polygonatum orientale, Medicago noeana* and *Cirsium vulgare* species were only present in the year following drought (Table 1).

Table 1. Changes in percent cover in relation to severe drought conditions in the Kurdistan region of western Iran

	Species	Family	Pre drought (2007 year)	Drought (2008 year)	After drought (2009 year)
1	*Astragalus sp.*	Fabaceae	24.37ab	18.37b	28.14a
2	*Heteranthelium piliferum*	Poaceae	1.5a	1.12a	2a
3	*Eryngium caucasicum*	Apiaceae	3.57b	2.55b	10.33a
4	*Psathyrostachys Fragilis*	Poaceae	2.97	0	0
5	*Echinops Haussknechtii*	Asteraceae	3.46ab	1.75b	7.5a
6	*Gundelia Tournefortii*	Asteraceae	8.46a	3.42b	6.78ab
7	*Euphorbia aucheri*	Euphorbiaceae	13.9a	4.92b	9.78ab
8	*Carex sp.*	Cyperaceae	1.08a	0	0
9	*Anthemis persica*	Asteraceae	6.35a	0	6.31a
10	*Phlomis kurdica*	Lamiaceae	13.18b	5.57c	18.65a
11	*Ferula Haussknechtii*	Apiaceae	10.76a	2.38b	8.53a
12	*Cynodon dactylon*	Poaceae	1.83a	.375b	2.75a
13	*Poa pratensis*	Poaceae	3.66a	0	1.02b
14	*Onobrychis andalanica*	Fabaceae	6.62ab	3.63b	7.9a
15	*Dactylis glomerata*	Poaceae	1.75a	1.12a	1.46a
16	*Lotus Gebelia*	Fabaceae	2.12a	0	2.5a
17	*Bromus tectorum*	Poaceae	1.45ab	.53b	2.79a
18	*Thymus kotschyanus*	Lamiaceae	4.23a	.27b	4.36a
19	*Tragopogon buphthalmoides*	Asteraceae	4.01a	.4b	2.57a
20	*Stachys lavandulifolia*	Lamiaceae	5.3a	5.48a	5.04a
21	*Grammosciadium platycarpum*	Apiaceae	4.25a	0	2.33b
22	*Falcaria falcarioides*	Apiaceae	3.1a	0	0
23	*Cardaria draba*	Cruciferae	0	0	.375
24	*Cicer anatolicum*	Fabaceae	.84a	.58a	1.31a
25	*Festuca ovina*	Poaceae	1.62a	0	0
26	*Mentha longifolia*	Lamiaceae	1.87a	0	1a
27	*Agropyrum kosaninii*	Chenopodiaceae	3.05a	2.3a	3.14a
28	*Achillea kellalensis*	Asteraceae	6.44a	4.24a	4.26a
29	*Vicia koeieana*	Fabaceae	2.4a	.65b	3.9a
30	*Glycyrrhiza glabera*	Fabaceae	1.14a	0	1.05a
31	*Plantago atrata*	Plantaginaceae	.55a	0	.68a
32	*Rapistrum rugosum*	Cruciferae	6.47a	0	2.29b
33	*Trifolium resupinatum*	Fabaceae	9a	0	7.5a
34	*Rheum Ribes*	Polygonaceae	7.53a	.87b	5.61a
35	*Scariola orientalis*	Asteraceae	2.2a	0	0
36	*Glaucium contortuplicatum*	Papaveraceae	7.66a	.33b	4.28ab
37	*Allium hitifolium*	Liliaceae	4.01a	.65b	5.31a
38	*Kelussia odoratissima*	Apiaceae	3.08a	.25b	3.23a
39	*Echium amoenum*	Boraginaceae	0	0	2.37
40	*Polygonatum orientale*	Liliaceae	0	0	1.75
41	*Medicago noeana*	Fabaceae	0	0	6.26
42	*Cirsium vulgare*	Asteraceae	0	0	1.5

Plant diversity varied significantly due to drought as shown by the Shannon-Wiener diversity index and Margalef richness index. Diversity decreased significantly in 2008 (drought year) and had a significant increase in 2009. The Smith-Wilson evenness index had no significant difference between years. Production was significantly reduced in 2008 but in 2009 increased significantly (Fig. 2-5).

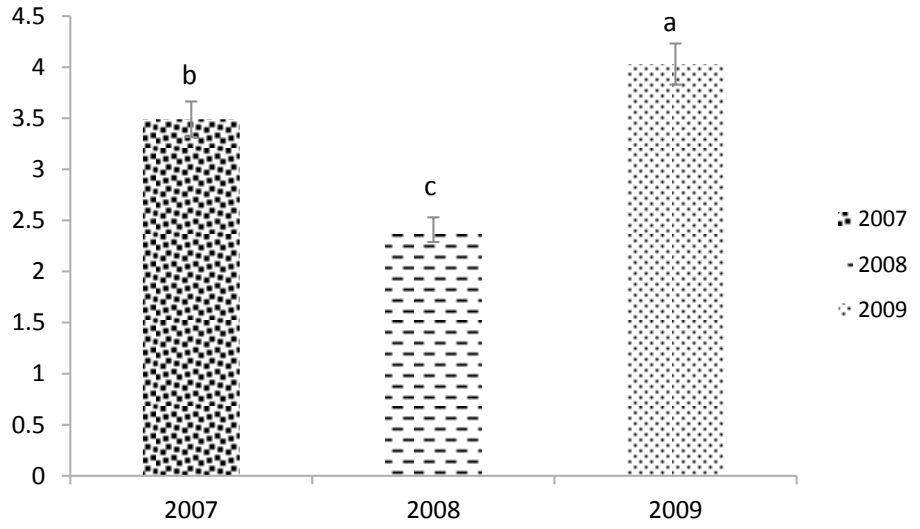

Fig.2. Margalef index measured before, during, and after a severe drought in 2008

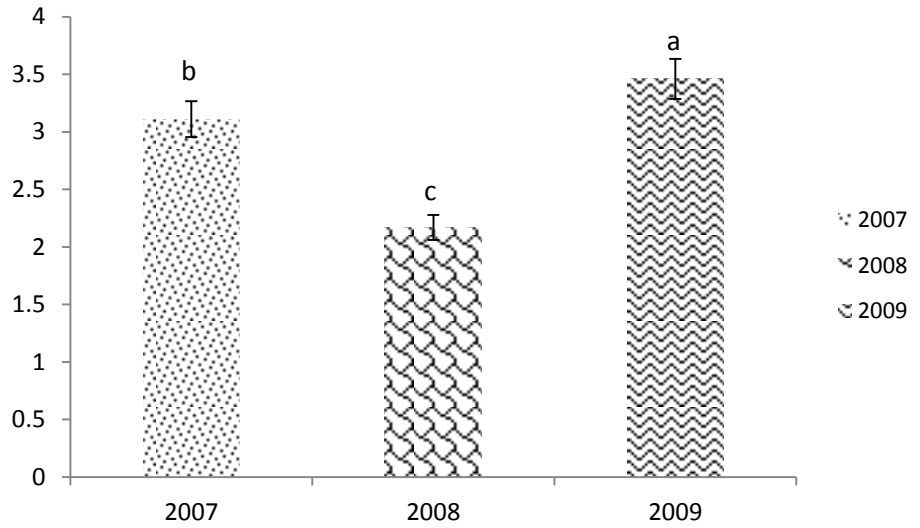

Fig.3. Shannon-Wiener index measured before, during, and after a severe drought in 2008

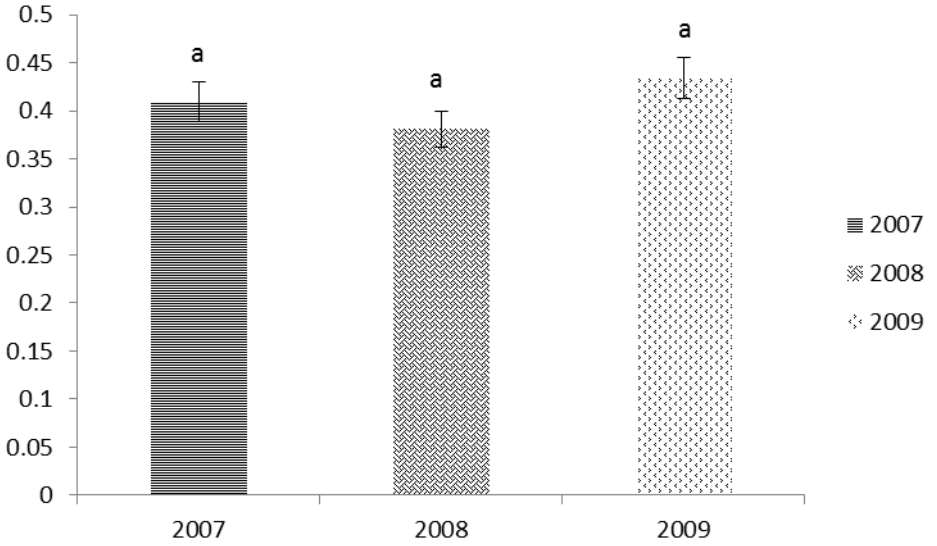

Fig.4. Smith- Wilson index measured before, during, and after a severe drought in 2008

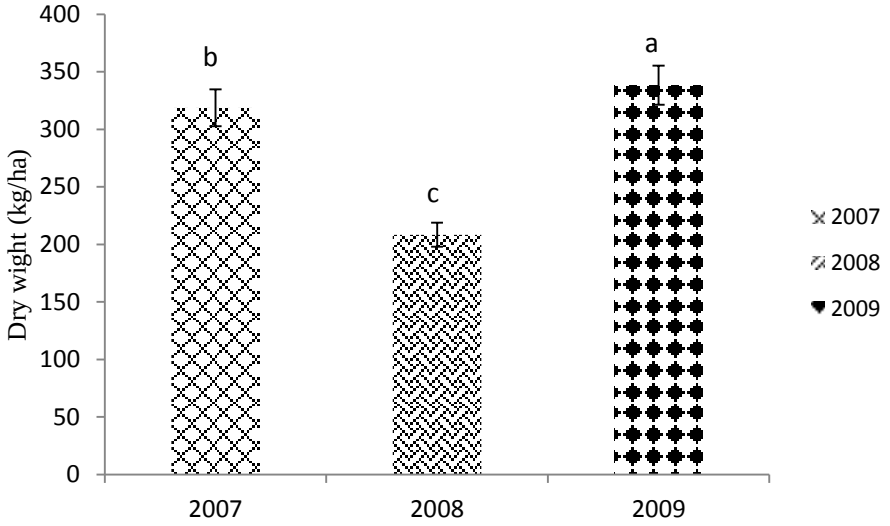

Fig.5. Dry weight (kg/ha) measured before, during, and after a severe drought in 2008

Discussion

Drought decreased species richness and diversity and reduced total plant ground cover. Drought affects many important plant processes, such as photosynthesis. Other studies had similar results (TILMAN & EL HADDI, 1992; HARTE & SHOW, 1995; KNAPP, 2002; MORECROFT, 2004; STAMPFLI & ZEITER; 2004; LLORET *et al*., 2009). As a direct consequence of drought, species composition might shift, productivity and reproduction could be reduced, and mortality increased (JENTSCH & BEIERKUHNLEIN, 2008). A possible indirect effect of drought could result from decreased vitality, making some species susceptible to damaging pathogens and insects (VOLNEY-LOUSTAU *et al*., 2006). Drought may also act indirectly through increased fire frequency, or by fires occurring where it was not previously common, affecting species poorly adapted to fire with significant negative ecosystem impacts. LINDNER (2010) found that areas at higher elevation could become drier and therefore more susceptible to fire, and might be a factor in the present study area, which is a high elevation site.

While elevated temperatures are expected to enhance soil fauna activity and decomposition rates, drought may counteract these effects or even lead to local extinction of some soil organisms (HULME, 2005). A strong link between herbaceous plant diversity and soil parameters associated with the availability of nutrients has indeed been reported in previous studies (RAMOVS & ROBERTS, 2003; CHUST *et al*., 2006; MARKS *et al*., 2008; BAI *et al*., 2011). Drought may affect soils due to altered soil moisture and litter decomposition rates (LINDEDAM *et al*., 2009). Drought in this region can indirectly affect species richness via altering soil water availability. Changes in soil moisture and temperature influenced processes such as litter decomposition, nutrient cycling, primary productivity, plant recruitment, survival, and the rate and direction of succession (COUTEAUX *et al*., 1995).

Some of the effects of the 2008 drought observed in this study were transitory, with a significant recovery in species richness and diversity occurring in 2009. MATIAS *et al*., (2011) showed that plant communities growing under wet conditions can have higher species richness and diversity. Other studies had similar results (STERNBERG *et al*., 1999; ADLER & LEVINE, 2007; ZAVALETA, 2003; YANG *et al*., 2011). As noted by SERENGIL *et al*. (2011), changes in precipitation and temperature in a region directly affect evapotranspiration, a key parameter in soil water budgets. Iran's arid to semi-arid climate means that water availability is one of the predominant limiting factors directly affect species richness by impacting the establishment and growth rates of species (BAZZAZ, 1996; NIU *et al*., 2008). In comparison to species richness, drought and rainfall had no effect on species evenness. YANG *et al.* (2011) similarly found that drought and precipitation had no effect on species evenness.

Productivity was significantly reduced in the year of drought and increased the following year under more normal precipitation levels. It is well known that that precipitation increases plant production but drought decreases it (SALA *et al*., 1988; BOLLINGER *et al*., 1991; LAUENORTH & SALA, 1992; PARTON *et al*., 1944; DHILLION & ANDERSON, 1994; KAHMEN *et al*., 2005; VAN RUIJVEN & BERENDSE, 2010). BOLORTSETSEG & TUVAANSUREN (1996) showed that increased precipitation enhanced plant biomass and prolonged the growing season.

Drought significantly reduced ground cover of sixteen species. These species were more sensitive to water shortage with the result that their regional abundance could be largely diminished in the event of widespread, long-term drought. One of the effects of climate change on biodiversity is increasing vulnerability of species (VOS *et al.,* 2008). Plants on nutrient-poor sites are more likely to suffer nutritional deficiencies with drought as nutrient uptake is highly correlated with water availability (MILAD *et al.,* 2011). GILGEN & BUCHMANN (2009) concluded that sites with lower annual precipitation seem to be more vulnerable to drought than sites with higher annual precipitation.

Sixteen species significantly increased with increasing rainfall. Nine species disappeared during drought, but reappeared after precipitation. In fact, drought eliminated these species, but seed of these species that was present allowed them to return when moisture conditions improved.

The most drought sensitive species were *Psathyrostachys fragili, Carex sp, Falcaria falcarioide, Festuca ovina* and *Scariola orientalis*. The drought caused local extirpation of these species and by eliminating their seeds and they failed to reappear when the one-year drought ended. All species that were extirpated had low abundance prior to the drought. If species are not able to reach new suitable habitat and fail to adapt to changing conditions, range loss and species extirpation are possible (ENGLER *et al*., 2009). Species with limited distributions are likely to be more prone to extinction due to climate change because gene flow between populations and colonization rates can be low (HAMRICK, 2004). BAKKENES *et al*., (2006) concluded that future climate change will exacerbate the

loss of species, especially those with strict climate and habitat requirements and limited migration capabilities. Environmentally extreme conditions such as severe drought enhance the probability of extinction of less abundant species (WHITE *et al*. 2000; LANTA *et al*., 2012). According to LLORET *et al*., (2004), species loss due to climatic alterations is related to species abundance, that is, less abundant species being more prone to disappear under drier conditions.

Cardaria draba, Echium amoenum, Polygonatum orientale, Medicago noeana and *Cirsium vulgare* species were not present before and during drought. Interestingly, drought provided an opportunity for these species to in-migrate from neighboring regions to successfully compete with existing species. Thus, while drought may affect current species composition, it appeared to also provide opportunities for plant migration, which may be an important natural mechanism to maintain net primary productivity and species diversity as the climate changes. Species which are unable to shift their range to higher altitudes are expected to be replaced as more competitive species are able to exploit higher elevations due to climate warming (VERBOOM *et al*., 2007). Entered species could affect ecosystems for example by competition, hybridization, diseases or altering habitats, culminating in extinction of some species and losses in biodiversity (HAMRICK, 2004).

Several species (*Heteranthelium piliferum, Dactylis glomerata, Stachys lavandulifolia, Cicer anatolicum, Aropyrum kosaninii* and *Achillea kellalensis*) were unaffected by either drought or drought recovery. This is believed due to the strong root systems of these species, enabling them to obtain water from deeper in the soil, and thereby avoid drought.

Conclusion

We document the reductions in plant species diversity and productivity in the forests of western Iran due to a severe regional drought. Iran is already one of the world's more arid countries, with only the north of the country having adequate precipitation. The 2008 drought raised significant concerns within Iran and by the FAO; due to the risk that increasing drought may lead to desertification. In the event climate change increases the incidence and severity of drought in Iran, it is important to begin planning for adaptation by conserving water and using it in ways that meet the ecological and social needs for water.

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Dispersion Modeling of the Air Pollution, Emitted by the Traffic in the Transport Tunnel under the Old Town of Plovdiv, Bulgaria

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Abstract. Air quality control and management is one of the areas in which Europe has been the most active in recent years. Air quality assessment is the process of determining the nature of ambient air pollution using monitoring and supplementary techniques such as modelling. Aim of this study was to evaluate the air pollution as a result of the traffic in the transport tunnel of "Tsar Boris III Obedinitel" Boulevard, situated under the Architectural Reserve "Old Town" in the central part of Plovdiv. Analyses included two consequent steps: (i) calculating the emissions of exhaused gases from the motor vehicles in the tunnel using the dispersion modeling software; (ii) comparing the values obtained with data from the automatic measuring station of the air quality, located in proximity of the tunnel. Our study revealed that the main air pollutant in the tunnel is the nitrogen oxides whose concentrations were 4 to 6 times higher than TLV. Carbon oxide concentrations were 5 times lower than TLV and those of the soot – two times lower. Curves of the NO_x, CO and dust concentrations changes in both cases (modelled and measured, respectively) are parallel which confirms the validity of the model used.

Key words: dispersion modelling, air pollution, traffic, street tunel, monitoring, Plovdiv

Introduction

Urban ecosystems are comprised of diverse land uses including commercial, industrial, residential, transport, recreational, agricultural and nature areas, resulting in different habitats within the urban landscape (PETROVA, 2011). Air pollution could be defined as a presence in the air of various gases, vapors, particles, solid or liquid substances, which adversely affect living organisms in the urban habitats, worsen environmental conditions or cause property damage. City of Plovdiv, Bulgaria, with a population of over 338 000 inhabitants (NSI, 2011), is subject to air pollution, often exceeding the threshold limit values (TLV) according to the current national standards (Regulation Norm 9, 2010), mainly by dust, however, by nitrogen oxides as well, caused by the transport (ATANASSOV *et al*., 2006).

Urban automobile transport is one of the major emitters of contaminants in the environment. Each car throws into the atmosphere large number of pollutants consisted of over 200 different compounds. These are primarily dust, carbon oxides, nitrogen and sulfur oxides, ozone, trace elements, benzene, toluene and other polycyclic aromatic hydrocarbons (PAHs). Many studies in different European cities have shown that motor vehicles are responsible for 75% of emissions of nitrogen oxides, 40% of carbon monoxide, 13% solids

and 3% of sulfur oxides (MONACI *et al*., 2000; ALLEN *et al*., 2001; HARRISON *et al*., 2003; HARRISON *et al*., 2004; WÅHLIN *et al*., 2006).

Air quality control and management is one of the areas in which Europe has been the most active in recent years. Air quality assessment is the process of determining the nature of ambient air pollution using monitoring and supplementary techniques such as modelling. Validation of model results against local monitoring data (if available) is eligible. Mathematical modelling of air pollution effectively enlarges our information about the air pollution level. It is an efficient tool for the elaboration and control of projects for air sanitation in areas with the individual extra protection (SZABO *et al*., 2005).

City transport tunnels represent a specific concentrator of pollutants in the air, especially in places with intensive traffic and moderate traffic speed. Transport tunnels can be compared to horizontal chimneys with two opposite emitting openings which in city conditions contribute substantially to the air pollution and in this role they focus the scientific attention of a number of authors (BELLASIO, 1997; YASSIN *et al*., 2005; SIMMONS & SEAKINS, 2012).

Aim of this study was to evaluate the air pollution as a result of the traffic in the transport tunnel of "Tsar Boris III Obedinitel" Boulevard, situated under the Architectural Reserve "Old Town" in the central part of Plovdiv. Analyses included two consequent steps: (i) calculating the emissions of exhaused gases from the motor vehicles in the tunnel using the dispersion modeling software; (ii) comparing the values obtained with data from the automatic measuring station of the air quality, located in proximity of the tunnel.

Materials and Methods

Study area. The road tunnel on "Tsar Boris III Obedinitel" Boulevard in Plovdiv under the Architectural Reserve "Old Town" was built in the 50-ies of the past century. It is a two-way road tunnel (two lanes for each direction), with North-South orientation (N 42.148515°, E 24.750755°), 183 m above sea level. Its dimensions are as follows: Length=216 m, Width=18 m, Height=5.1 m, Cross-section=105 m^2 (i.e. volume of 22680 m^3).

On a busy juncture nearby the tunnel – 460 m away from its northern side, operates the automatic measuring station „Banya Starinna"(AMS) for the air quality monitoring (N 42.152641°, E 24.750736°), which allowed us to compare the air pollutants' concentrations – NO_x, CO and dust particles (soot), calculated with the dispersion model to the data for the same pollutants, measured by the AMS (Fig. 1).

Selection of place and time to conduct the counting. In order to evaluate the emissions by the motor vehicles (MV) in the tunnel, an observation point for counting the vehicles was set up, located above the northern side of the tunnel which revealed a sufficiently long part of the boulevard to the observer (hundreds of meters). This viewpoint gave the counters sufficient time for unambiguous classification into 13 categories of the motor vehicles, passing through the tunnel.

Study was conducted in two successive, sunny, dry and warm for the season days, without any need of domestic heating at least, in the middle of the day.

Counting was carried out in the following one-hour time intervals:

- from 17 hour 15 minutes to 18 hour 15 minutes on March 14, 2013, Thursday. Our expectations, based on preliminary observations, were that this time interval corresponded to the evening maximum of the MV number. In the text below this interval is abbreviated as "17-18 h".
- from 8 hour 20 minutes to 9 hour 20 minutes on March 15, 2013, the interval of the expected morning maximum of the MV number, expressed in short as "8-9 h".
- from 12 hour 16 minutes to 13 hour 16 minutes on March 15, 2013 – the middle of the work day. The interval is briefly expressed as „12-13 h".

Counting of the motor vehicles. For the purposes of the study, a suitable software was developed (by Nikolay Takuchev) for traffic counting (using Visual Basic for Applications, build in MS Excel). It allowed us both to easy and quick register the MV passing through the observation point, and

to classify them into categories by the mouse click on the button in the software window, corresponding to the passing motor vehicle (Fig. 2).

Computers saved a database with the type of each MV and its moment of passing with precision of a second. Only quota of the categories 4 and 8 was calculated according to the statistical data from the Ministry of Internal Affairs of Bulgaria (2013).

Dispersion modelling procedure.

Handbook of assessment and management of ambient air quality at the local level for SO_2, PM_{10}, Pb, and NO_2 (MOEW, 2002) was elaborated on the basis of the Joint EMEP/CORINAIR Atmospheric Emission Inventory Guidebook (EEA, 2001) by the Ministry of Environment and Waters in Bulgaria.

Fig. 1. Satellite picture of the transport tunnel in the city of Plovdiv, Bulgaria (source Google Earth, 2014), location of the observation point and of the automatic measuring station (AMS)

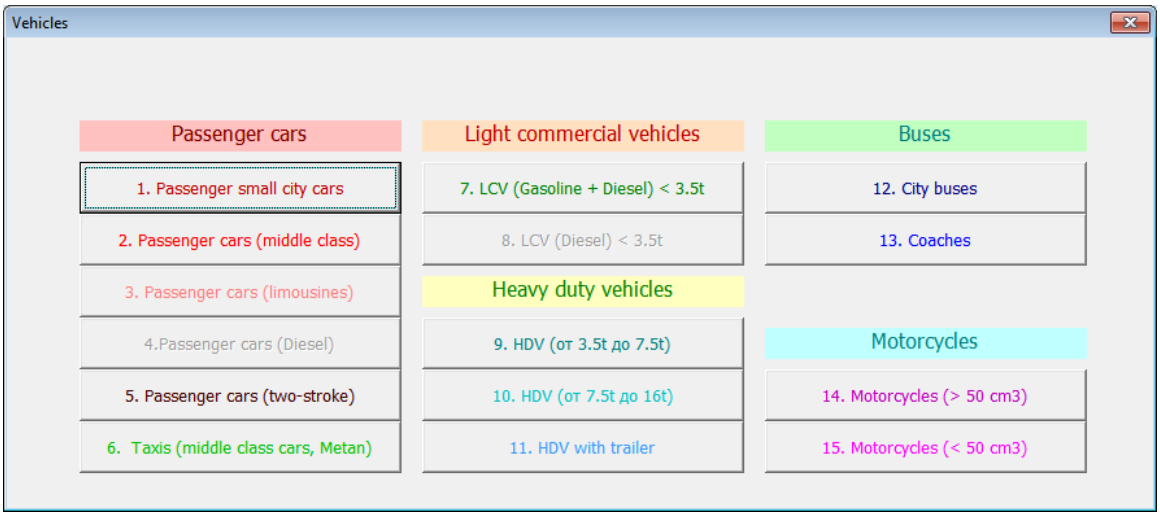

Fig. 2. Screen window of the software for counting of the 15 categories MV.

On the basis of this methodology that is intended for mathematical modelling of air pollution an user-oriented software product, named Traffic Oracle Program Software, was developed by the Geophysical Institute of the Bulgarian Academy of Sciences on 2004 (Traffic Oracle Program Software, 2002). This model for the ground surface field determines the concentrations of gaseous pollutant substances in the air and the solid particles to 20 micrometers. It makes possible to calculate short and long-term critical concentrations of pollutants and the overfull of threshold values. It was spread according to the methodology US EPA - ISC.

Given the source strength, meteorology, site geometry, and site characteristics, the model can reliably predict pollutant concentrations for receptors located at the chosen points. In this study, the model presents only pollutants such as carbon monoxide, nitrogen oxides and particular matter (PM).

In order to assess the MV emissions in the ambient air, the motor vehicles passing through the observation point, shall be distributed according to the type of their engines into 15 categories (13 counted and 2 calculated by statistical data). The MV categories were determined according to the SNAP nomenclature (Selected Nomenclature for Sources of Air Pollution) of EMEP/CORINAIR (European Monitoring and Evaluation Programme/The Core Inventory of Air Emissions in Europe) (EB.AIR/GE.1/1997/5).

Due to the dependence of the emissions on the age of the engines, the MV number in each of the 15 categories was divided into 4 subcategories according to the age of the engines, i.e. separately for each one of time intervals. Statistical data about the percent distribution of the motor vehicles according to type and age of the engines were obtained from the Ministry of Internal Affairs of Bulgaria (2013).

In order to count and classify quickly and precisely the motor vehicles in the 15 mentioned categories, the counters carried out a preliminary counting and training – both for the quick identification of the MV categories and for handling the software, specifically developed for the counting. Counting was performed by two counters using portable computers – one for each direction in the tunnel.

In the beginning and at the end of each one-hour counting interval, the temperature, the velocity and the direction of the wind (VORTEX Hand Held Wind Meter) through the tunnel were measured. Ambient temperature plays a role in the calculation of the traffic emissions while the wind power and direction determine the calculated pollutants concentration.

Calculation of the emission factor (EF) – the mass of a specific pollutant, emitted in the air from all MV per unit time and per unit length (unit g/s/m) was performed with the Emission module of the Traffic Oracle Software. Calculation of the traffic-emitted pollutants concentrations was performed with the Diffusion module of the same software.

Statistical evaluation. Student test was used for testing of the calculated concentrations by dispersion modeling and measured concentrations by the AMS, respectively for each one of studied pollutants ($p<0.05$). Relationships between two types of data were tested using Pearson correlation coefficients ($p<0.05$). All statistical analyses were made with the STATISTICA 7.0 statistical package (STATSOFT, 2004).

Results and discussion

Traffic counting characteristics

Traffic past was intensive and continuous - a total of 8745 motor vehicles passed through the observation point for the three time intervals. Distribution of the MV number into the three studied one-hour intervals is presented in Table 1.

We revealed, that ¾ from all motor vehicles, passing through the tunnel, were medium class passenger cars, (2^{nd} + 6^{th} category). Number of some MV categories increased during the day – that of taxis, limos and small city cars. At noon, the number of middle-class cars and buses had

minimum and the number of light commercial vehicles – maximum. Total number of vehicles was also increasing throughout the day.

Table 1. Distribution of the counted motor vehicles, passing through the tunnel under the Architectural Reserve "Old Town" in Plovdiv (Bulgaria).

	17-18 h on 14 March 2013	**8-9 h on 15 March 2013**	**12-13 h on 15 March 2013**	**Proportion according to the MV type, %**
1. Passenger small city cars	208	159	174	**6**
2. Passenger cars (middle class)	2615	1525	1477	**64**
3. Passenger cars (limousines)	389	107	213	**8**
6. Taxis (middle class passenger cars)	410	270	310	**11**
7. Light commercial vehicles < 3.5t	105	172	211	**6**
12. City buses	138	139	123	**5**
Total	3865	2372	2508	
Proportion, %	**44**	**27**	**29**	

Matrix for determination of the emissions

Categories 4 and 8 (diesel cars and light commercial vehicles) were calculated according to the total number of counted vehicles of the respective type and the statistical data (Ministry of Internal Affairs, 2013), according to which the passenger diesel cars are 20%, while the light commercial vehicles are 80% of total number of cars in the country.

According to the mentioned method, in addition to the MV type, they had to be divided according to their age into 4 categories: above 20 years; between 15 and 20 years; between 10 and 15 years; less than 10 years. Data about the age distribution of the motor vehicles were obtained by the Ministry of the Internal Affairs (as on 31.12.2010) (Table 2).

Data from Table 1 and Table 2 were set in the software re-distributing the MV number, obtained after counting, into 60 categories according both to the type and engine age.

Slope of the street has an impact on the traffic emissions as the ascending cars burn more fuel than the descending ones. Slope of the road section through the tunnel is small (about 1%), and was ignored in the calculation of the emissions. MV flows in the two opposite directions were treated as one common flow.

Average temperature values, wind power and direction through the tunnel for the three time intervals are presented in Table 3.

Emission factors of the pollutants were determined using the Emission module of the Traffic Oracle Program Software (2002).

As input parameters in the Emissions module for the calculation of the emission factors of the nitrogen oxides, the carbon monoxide and the dust particles (soot) for each of the above-mentioned time intervals, were used:

- MV distribution into 60 groups (as described above)
- Road type – the option "a town street" was chosen,
- Road slope – 0% (no slope),
- Air temperature – data from Table 2,
- Counting time – 60 minutes,
- Type of pollutant – nitrogen oxides, carbon monoxide, and soot (part of the dust particles pollution), respectively.

Three emission factors were obtained for each one of the three time intervals and were presented in Table 4.

Determination of the pollutants concentrations

In order to determine the air pollutants concentration along the tunnel length, the following three data groups were included in the Diffusion module of the Traffic Oracle Program:

Table 2. Distribution of MV according to their age.

	Proportion, %			
	above 20 years	***between 15 and 20 years***	***from 10 to 15 years***	***less than 10 years***
Passenger cars	25.46	32.63	29.17	12.74
Light commercial vehicles	20.68	25.89	26.02	27.42
Heavy duty vehicles	41.49	22.13	17.82	18.56
Buses	36.53	24.77	18.99	19.71
Motorcycles	29.74	17.17	18.62	34.47

Table 3. Temperature and wind velocity values during counting (measured at 1.8 m above the road pavement, 1 m away from the wall at the tunnel entry).

Time interval	**Temperature, °C**	**Wind velocity magnitude, m/s**	**Wind velocity direction**
8-9 h	13.8	3	South
12-13 h	16.5	2.5	South
17-18 h	18.6	6	South

- *Input model parameters*

This module calculates the pollutants concentrations in the nodes of a rectangular grid on the map. Density and dimensions of the grid are preset by the user who determines the number and step sizes to the East and North. As disposition of the tunnel is in North-South direction, the step size in the grid was chosen North-Southwards 10 m and the number of the steps was North-South = 24, i.e. the calculation of the concentrations was executed for length of 240 m – 10 m outside the two ends of the tunnel. Chosen number of the steps West-Eastwards were equal to two, with 10 m step size.

Mobility of the pollution depends on the type of the underground surface – in this case the "city region" option was preferred.

- *Meteorology*

In the beginning and at the end of the counting intervals the air temperature was measured at 1.8 m and at 7.8 m above the tunnel floor. Temperature difference for the three intervals was about 0.2°C, i.e. the vertical temperature gradient in the three cases was similar by magnitude and close to the neutral stability in regards to the vertical motions in the atmosphere, which was preset in the calculations. The option "one wind direction" was selected - South (Table 3).

- *Source parameters*

Ambient air pollution due to a linear source (the MV flow through the tunnel) was calculated with input parameters the coordinates of the tunnel ends – from 10 m to 230 m, and linear source width (the street) -18 m. Gravitational deposition velocity of the gas emissions was set as 0 m/s, for the soot – as 0.07 m/s (EMEP/CORINAIR Atmospheric Emission Inventory Guidebook, EEA, 2001). Input parameter of the source correlated also with the emission factor for the respective pollutant and the time interval (Table 4).

Concentrations of the Nitrogen oxides

Concentrations of nitrogen oxides along the axis line of the street in the tunnel, emitted by the traffic during the three MV counting intervals, were shown on Fig. 3. For the three measurement intervals, the NO_x concentrations have growing to the wind-blown end of the tunnel. Negative relationships were found between the NO_x concentration, and the air temperature and wind velocity – the NO_x level was about 3 times lower when the wind's speed increased two fold and the temperature

increased with 6°C ($p<0.05$). Threshold limit value (TLV) of 200 µg.m^{-3} (Regulation Norm 9, 2010) was exceeded in all three studied time intervals.

Next step was to compare the calculated average concentration of NO_x on the tunnel axis with the concentration, registered by AMS „Banya Starinna“ at the same time interval (Fig. 4). Since the street through the tunnel passes also besides the AMS „Banya Starinna“, located 460 m away, i.e. almost all cars, passing through the tunnel pass also besides the station, proportionality of the concentrations in the tunnel pollution should also be expected in the region of AMS. Statistical evaluation of two types of data found no significant difference ($p<0.05$). Almost parallel disposition of the lines in Fig. 4 corresponds to this conclusion; therefore, it is an indirect proof for the validity of the nitrogen oxides concentrations in the tunnel air, calculated with the dispersion model. This comparison yields the conclusion that the nitrogen oxides concentration in the tunnel (at the level of the car exhaust-pipes, along the axis line of the street) is about 4.5 times higher than in the open air around the AMS „Banya Starinna“ (30 m away from the street, at 3 m above the street pavement – the height for sample taking by the analyzers of the automatic station).

Concentrations of the Carbon oxide

Calculated carbon oxide concentrations in the tunnel are presented on Fig. 5. Pollution distribution along the tunnel axis was found to be with the same form as that of the nitrogen oxides; however, the concentrations of CO remained about 5 times lower than TLV in all studied time intervals. Dependence of the pollution level from the wind and temperature was similar to that of the nitrogen oxides – it decreased when the wind became stronger and the temperature increased ($p<0.05$).

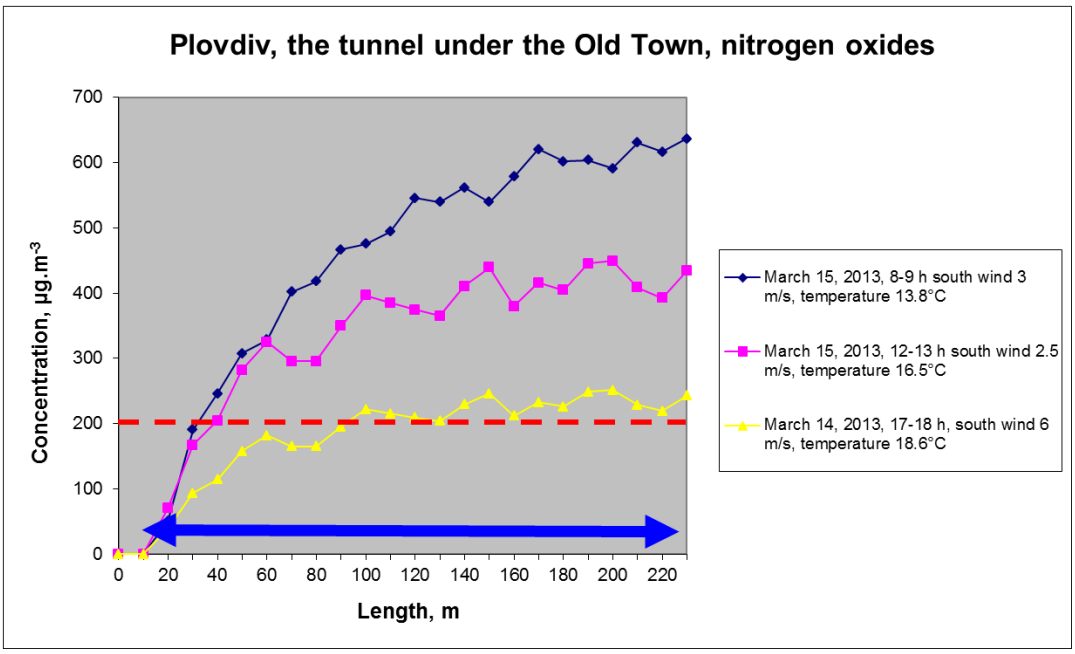

Fig. 3. Dynamic in the nitrogen oxides concentrations along the axis line of the tunnel under the Old Town in Plovdiv (Bulgaria). Double arrow showed the tunnel length; dashed line - the threshold limit value (TLV).

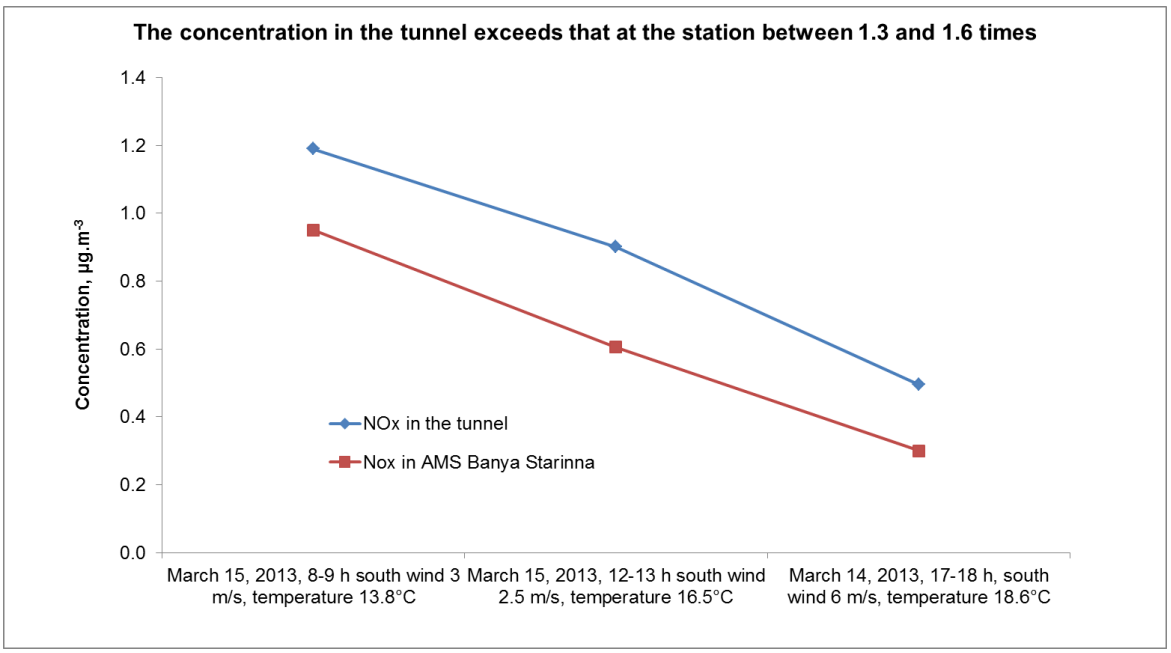

Fig. 4. Comparison between the nitrogen oxides concentrations calculated for the tunnel and registered by AMS „Banya Starinna", respectively.

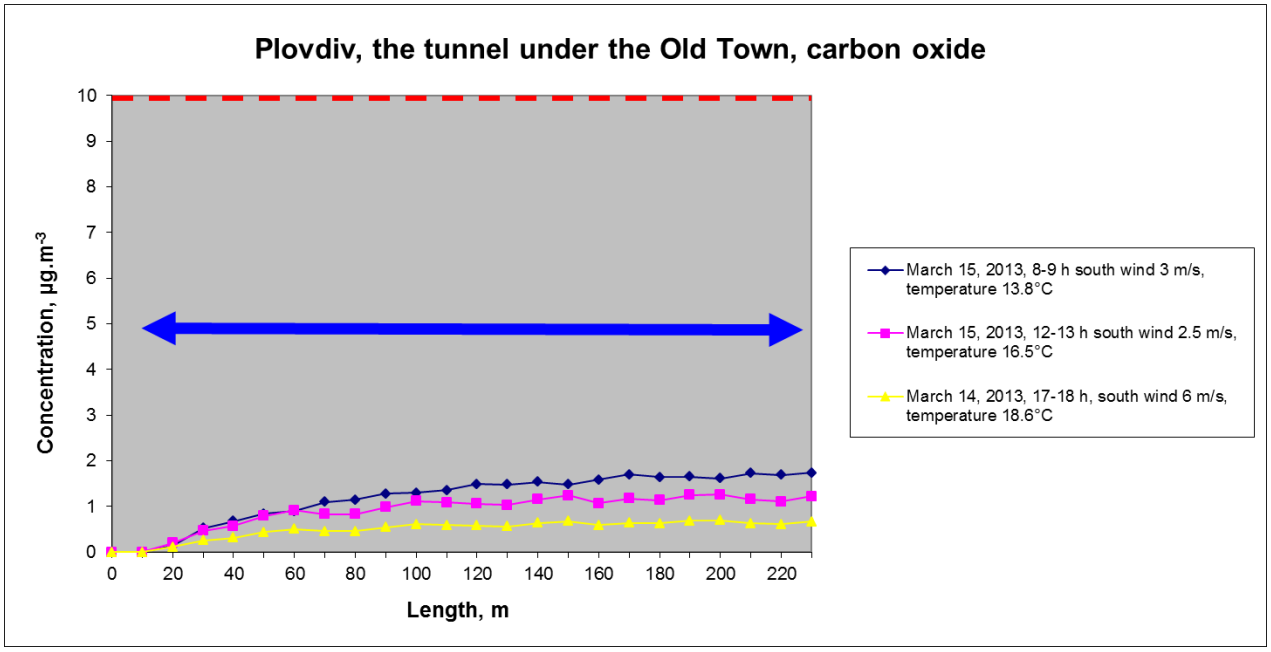

Fig. 5. Distribution of the carbon oxide concentrations along the axis line of the tunnel under the Old Town Plovdiv. A double arrow marks the tunnel length. A dashed line presents the threshold limit value (TLV).

When comparing the calculated by the Dispersion module values of CO concentration with those registered by the AMS, we found no statistical significant differences ($p<0.05$). Fig. 6 shows the simultaneous change of the concentrations – the average one, calculated along the tunnel length and those, registered by AMS „Banya Starinna". Curves of the concentration change in two cases are parallel which confirms the validity of the model used. Comparison leads to the conclusion that the carbon oxide concentration in the tunnel (at the level of the car exhaust-pipes, along the axis line of the street) was about 1.5 times more than in the open air (30 m away from

the street, at 3 m above the ground – the height for sample taking by the analyzers of the automatic station).

Concentrations of the Soot from the burning fuel

Calculated concentrations of soot as a result of the fuel burnt by the engines in the tunnel are presented on Fig. 7. These are part of the dust particles ejected in the air by the traffic. Distribution of the pollution along the tunnel axis was quite similar to those of the nitrogen oxides and the carbon oxide; however, the concentrations of soot remained approximately two times lower than TLV. Negative relationships of the soot pollution by the wind and air temperature were found, similarly to the tendency of the nitrogen oxides level.

Fig. 8 presents the common change of the both concentrations – the average soot level, calculated for the tunnel axis, and the PM_{10} content in the ambient air, registered by AMS. Concentration of the dust particles PM_{10} (particulate matter with aerodynamic diameter of 10 μm), registered with the automatic station and the calculated soot concentration were almost the same at noon, but significantly differed in the morning and evening when the air pollution outside the tunnel prevailed ($p<0.05$). This discrepancy between the observed and the calculated concentrations could be due to the emissions from domestic heating (during the experiment the heating season wasn't over yet) and to the increased dynamic atmosphere stability in the beginning and at the end of the day.

From the other hand, the particulate matter (PM) within the urban environment are often associated not only with exhaust emission, but also with tire, brake, vehicle and engine wear, or the re-suspension of road dusts (ALLEN *et al.*, 2001; HARRISON *et al.*, 2003; MONACI *et al.*, 2000; RIGA-KARANDINOS & SAITANIS, 2004; WÅHLIN *et al.*, 2006).

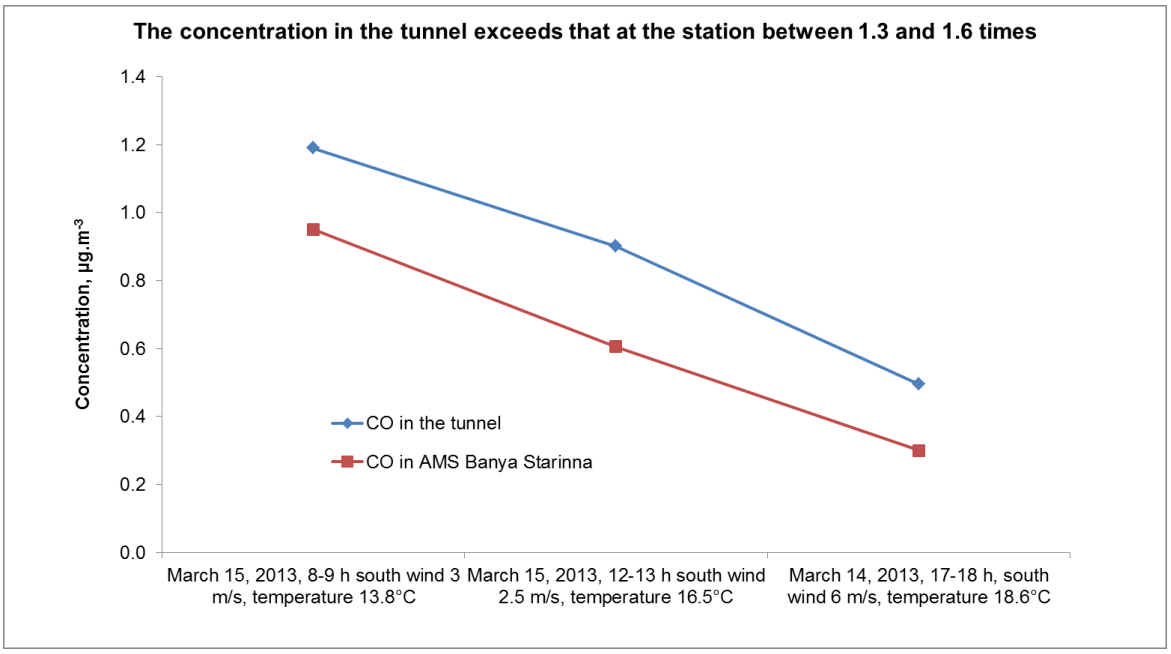

Fig. 6. Comparison between the carbon oxide concentrations – the one, calculated for the tunnel and the one, registered by AMS „Banya Starinna".

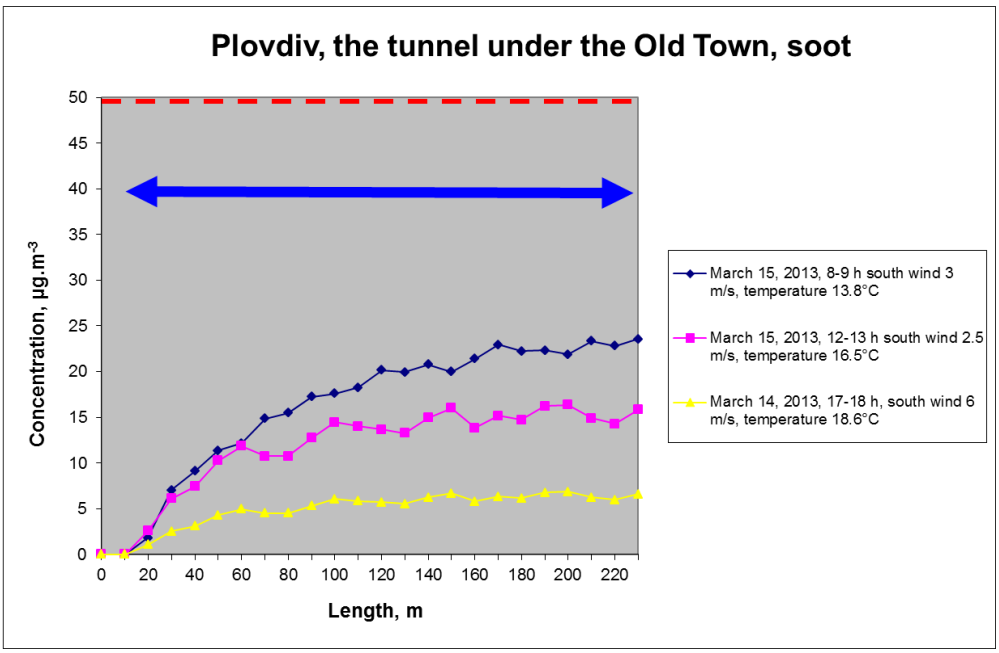

Fig. 7. Distribution of the soot concentration along the axis line of the tunnel under the Old Town Plovdiv. A double arrow marks the tunnel length. A dashed line presents the threshold limit value (TLV).

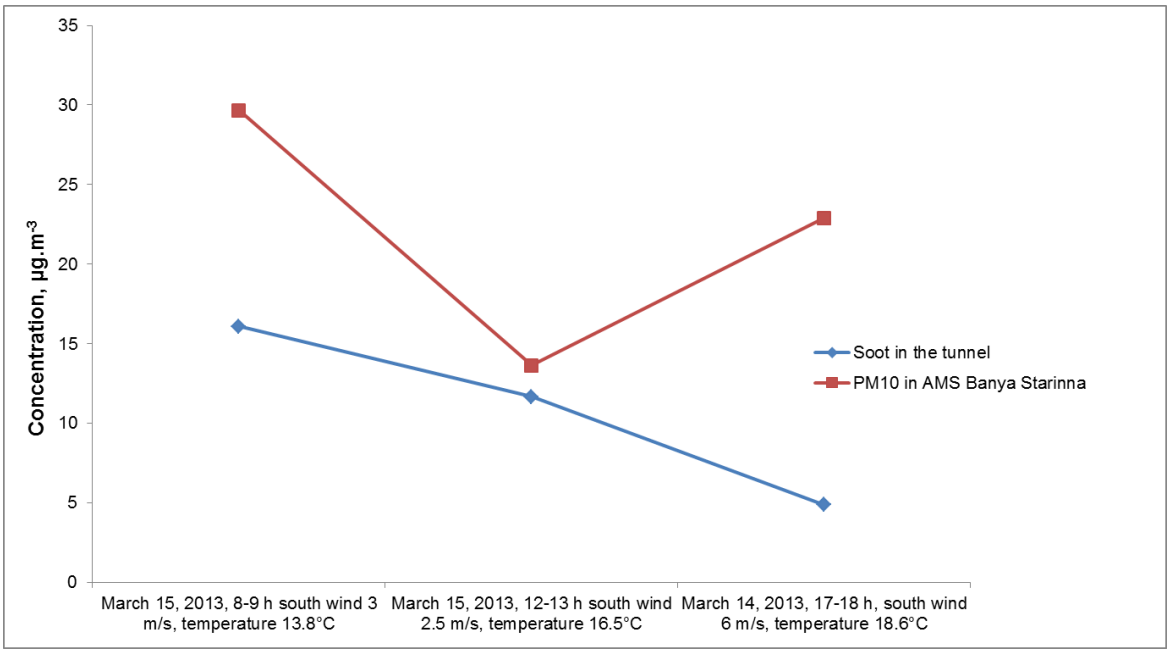

Fig. 8. Comparison between the concentrations of soot emitted in the air as a result of the engine combustion – the calculated concentration of soot by the engines in the tunnel and the concentration registered by AMS „Banya Starinna" differ in the beginning and at the end of the work day and almost coincide in the middle.

Average concentrations of NO_x, CO and Soot in the tunnel

Average concentrations of the studied air pollutants in the tunnel volume as a whole could be assessed by evaluation of the emitted pollutant mass in the tunnel, divided into its volume. Overall emitted pollutant mass of all MV in the tunnel could be calculated by multiplying the emission factor value to the time for MV passing through the tunnel. As the maximal permitted motion speed in city conditions is

50 km/h=13.9 m/s, and the tunnel length is 216 m, so the time for MV passing through the tunnel is 15.6 s.

Average pollutants concentrations in the tunnel were calculated as the emitted pollutants mass was divided to the tunnel volume (22680 m^3) and were presented in Table 5. These concentrations in the tunnel volume could be used only for orientation since the wind and the atmosphere dynamic stability have not been taken into account, and the complete mixing of the emissions with the air in the tunnel was presumed.

Table 4. Emission factors of the pollutants during the experiment.

Time interval	Emission factor, g/s/m		
	NO_x	*CO*	*Soot*
8-9 h	0.0010138	0.0027713	0.0000375
12-13 h	0.0010128	0.0028481	0.0000369
17-18 h	0.0013583	0.0037529	0.0000370

Table 5. Average concentrations of the studied pollutants in the tunnel.

	Average concentration		
Time interval	NO_x, $\mu g/m^3$	*CO*, mg/m^3	*Soot*, $\mu g/m^3$
8-9 h	134.2	0.37	4.96
12-13 h	134.1	0.38	4.88
17-18 h	179.8	0.50	4.90

Sources and characteristics of studied pollutants

By the recent decade we find ourselves in the middle of a period of rapid change in vehicle emissions. Improvements in vehicle and fuel technology have led to rapid reductions in the average rate of per vehicle emissions of CO and particulates. Emission modeling predicts that this reduction will continue, albeit at a reduced rate, for at least a further decade. Trends in the precursors of NO_2, however, are much less clear. There is emerging evidence that, in contrast to CO and PM, the trend is upwards. Whether all of this leads to improvement of the air quality depends on whether reduced emissions are offset by growth in traffic or increases in congestion.

Carbon monoxide emissions arise from incomplete or inefficient combustion. Petrol engines tend to emit greater amounts of CO than diesel. Technological advances have substantially improved CO emissions from newer vehicles, such that CO emissions from the vehicle fleet have rapidly declined over the last decade and reductions are expected to continue over the next decades.

Sources and determinants of **nitrogen oxides** in the tunnel are more complex. NO_2 can be emitted directly from vehicle tailpipes, but mostly indirectly via the emission of nitric oxide (NO). Diesel vehicles are the dominant source and the fraction of NO_x emitted directly as NO_2 is also greater for diesel vehicles. The dominant source of NO_2 is in-situ chemical formation from the reaction of NO with ozone. Ozone is a natural component of the atmosphere. The reaction between NO and O_3 to form NO_2 is rapid, especially in the dark. For this reason, emissions of NO suppress the availability of O_3 in urban areas. Other factors also influence O_3 levels, and on a short-time basis ambient ozone can vary rapidly and unpredictably in response to meteorological conditions.

The potential for NO_2 formation inside tunnel is likely to depend on the external levels of ozone and how far it can penetrate into the tunnel before it is depleted. However, even if there is zero available

ozone, NO_2 can still arise in the tunnel from two less significant sources: from external (ambient) sources or from limited direct emission from the tailpipe (particularly from diesel engines). All of these factors are complex and difficult to predict. In summary, however, there is greater potential for elevated NO_2 levels, relative to CO, in rural tunnel with high proportions of diesel vehicles.

Nitrogen oxides are pollutants associated with several adverse respiratory effects, especially in children. Asthmatics have been shown to be particularly vulnerable to these effects and have an increased sensitivity to particles and allergens subsequent to NO_2 exposure. Inhalation of nitrogen dioxide in the presence of airborne particulates can lead to respiratory symptoms in asthmatics at concentrations which can occur in some road tunnels. However, this finding is based on exposures of 30 minutes or more; the effect of shorter exposures is currently unknown. Long-term repeated exposure, as may be experienced by regular commuters, has been associated with reduced lung function and other adverse effects in children.

Fine particulates are derived from both vehicle tailpipe emissions, resuspension of road dust, tire and brake wear and from material lost from truck loads. There are substantial evidences for effects on respiratory health, risk for exacerbations of cardiovascular disease (including mortality) and cancer. However, the casual pathways linking exposures to these effects are still active areas of research. Most of the evidence is focused on exposures of hours, days or years, not seconds or minutes. Although it is toxicologically plausible that very brief exposures to very high levels of vehicle-related particulates (as is the case in a road tunnel) poses a risk to health, there is currently insufficient scientific evidence to quantify any risk and there are no associated guidelines.

External air quality

A roan tunnel has an impact on external air quality because nearly all of the air pollutants emitted by vehicles within the tunnel are, at some point, vented into the ambient atmosphere, either via the portals, and/or via one or more stacks. This has the potential to lead to localized degraded air quality and the potential for exceedances of national standards.

For a tunnel without stacks, contaminated air is vented at the exit portals. Tunnel portal emissions are complex and difficult to assess. However, the extent of the affected zone is typically of the order of 100-200 m. If this localized impact is too high, the tunnel air can be vented elsewhere, at a ventilation station, and possibly via a tall stack. In some cases this stack is at some distance from the tunnel, so that tunnel air may be vented into the atmosphere in a non-residential location. Stacks are shown as remarkably efficient at dispersing pollutants (COLBERG *et al*., 2005). Concentrations at ground level are strongly reduced when stacks are used and very tall stacks can have minimal to zero impact in their local vicinity. One of the great advantages of road tunnels is the opportunity to deliberately site portals (or stacks) away from sensitive receptors so that road transport emissions may be removed from dense residential areas improving local air quality.

Conclusions

The 24-hour cyclic recurrence in the city traffic determines cyclic recurrence in the concentrations of the main pollutants – nitrogen oxides, carbon oxide and dust. During the experiment, two peaks were outlined in the traffic intensity through the observation point above the tunnel – in the time intervals 8:40 – 8:50 h and 17:15 – 17:35 h.

Comparison of the calculated concentration of nitrogen oxides and carbon oxide from the tunnel traffic to the concentration of the same air pollutants, measured by the automatic station, showed that the changes in the concentration are synchronous and proportional. This fact corresponds to our expectations upon supposition that the pollution source in the studied area is the same transport flow,

passing both through the tunnel and along the station. Changes of the both dust particles concentrations, calculated for the tunnel and measured by the station, respectively, differ in the beginning and at the end of the work day, probably due to the domestic heating.

Our study revealed that the main air pollutant in the tunnel is the nitrogen oxides whose concentrations were 4 to 6 times higher than TLV. Carbon oxide concentrations were 5 times lower than TLV and those of the soot – two times lower.

Compared to a surface road or the ambient environment, the air quality as experienced by tunnel users is relatively poor, with the concentrations of traffic-related contaminants likely to be many times higher inside as opposed to outside the tunnel. The two main determinants of in-tunnel concentrations (emissions from the tunnel openings into the external environment) are the rate of vehicle emissions and the rate of ventilation. Vehicle emission rates are related to vehicle volume, speed, the proportion of heavy duty vehicles, and road gradient. Carbon monoxide arises from tailpipe emissions, whilst nitrogen dioxide arises indirectly from the chemical oxidation of tailpipe emissions. Particulates arise from both tailpipe emissions, resuspension of road dust, tire and brake wear and from material lost from truck loads. Oxides of nitrogen arise from tailpipe emissions, with a bias towards diesel engines relative to carbon monoxide.

In recent years improvements in fuel specifications and vehicle technology has led to rapid reductions in the emission rates of CO and PM of new vehicles. NO_x emissions not arise from the reaction of the two main constituents of the atmosphere (nitrogen and oxygen) in the high temperatures of combustion, rather than any component of the fuel. NO_x emissions rates respond differently to fuel and technology change. In recent years NO_x emissions rates have reduced much more slowly than for CO and PM, a trend which is expected to continue for some years to come.

Consequently, a campaign of high quality NO_x measurements, supported by simultaneous, detailed and continuous monitoring of traffic flow, speed and composition and air flow is recommended as a first priority. An interim objective should be to evaluate or adjust a tunnel-specific emission model for prediction of future ventilation demand.

Tunnel monitoring campaigns are critical for ensuring the all air quality management/air pollution mitigation measures, such as road tunnel ventilation systems, correctly to ensure they continue to provide the designed level of mitigation in order to meet objectives set in the Bulgarian legislation.

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Insecticidal Activity of Essential Oil from Juniperus communis L. subsp. hemisphaerica (Presl) Nyman against Two Stored Product Beetles

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Abstract. In the current study, insecticidal activity of essential oil from fruits of *Juniperus communis* L. subsp. hemisphaerica (Presl) Nyman was evaluated against *Rhyzopertha dominica* (F.) and *Tribolium castaneum* (Herbst) by fumigation at 24, 48, and 72 h exposure times. Dry fruits were subjected to hydrodistillation using a Clevenger-type apparatus and the chemical composition of the volatile oil studied by gas chromatography-mass spectrometry (GC-MS). The major components were identified α−pinene (59.70%), and limonene (9.66%). Insecticidal activity was varying with essential oil concentration and exposure time. Results showed that *R. dominica* is more susceptible than *T. castaneum* for all exposure times. LC_{50} values at 24 h were estimated 36.96 µl/l air for *R. dominica*, and 107.96 µl/l air for *T. castaneum*. These results suggested that *J. communis* subsp. hemisphaerica fruit oil may have potential as a control agent against *R. dominica*, and *T. castaneum*.

Keywords: *Juniperus communis* subsp. hemisphaerica, essential oil, fumigation, stored product beetle.

Introduction

Juniperus L. (Cupressaceae) is a genus of evergreen shrubs or trees and the second most diverse of the conifers, with some 67 species in the world (ADAMS, 2004). In Iran, Cupressaceae family consists of one species of *Platycladus*, one species of *Cupressus*, and five species of *Juniperus*. *Juniperus communis* L., *Juniperus excelsa* M. Bieb., *Juniperus foetidissima* Willd., *Juniperus oblonga* M. Bieb and *Juniperus sabina* L. are represented species (ASSADI, 1998).

Amongst the *Juniperus* L. genus, the most renowned species used in traditional medicine is *J. communis* (GAUTAM *et al*., 2007; GONZALEZ-TEJERO *et al*., 2008). Its dried bluish-black cones, known as "juniper berries", are said to stimulate the appetite and are used as a flavoring agent for culinary purposes and in the preparation of gin spirits (FOSTER, 1999; DARWIN, 2000). They have also been used for various medicinal purposes, including as an antiseptic, contraceptive, diuretic, and as a remedy for urinary tract infections, scrofula, chest complaints, diabetes, rheumatism and backache. The smoke from burnt juniper branches has been used as a fumigant to prevent the spread of infections (TILFORD, 1997; DARWIN, 2000; NEWTON *et al*., 2002; ALLEN & HATFIELD, 2004). In addition, there are numerous reports on the biological

activity of the essential oil of *J. communis* (EMAMI *et al*., 2007a, b; GORDIEN *et al*., 2009; MICELI *et al*., 2009; REZVANI *et al*., 2009).

The present work was carried out to identification of chemical compounds as well as determines the possible fumigant toxicity of the essential oil of the fruits of *Juniperus communis* L. subsp. hemisphaerica (Presl) Nyman against *Rhyzopertha dominica*, and *Tribolium castaneum*.

Material and methods

Insect culture

Rhyzopertha dominica and *T. castaneum* were reared on whole wheat and wheat flour mixed with yeast (10:1, w/w), respectively. Adult insects, 1–7 days old, were used for fumigant toxicity tests. The cultures were maintained in the dark in a growth chamber set at 27±2°C and 60±5% r.h. Parent adults were obtained from laboratory stock cultures maintained at the Entomology Department, University of Urmia, Iran. All experiments were carried out under the same environmental conditions.

Plant material

The fruits (berries that formed in the current year) of *J. communis* subsp. hemisphaerica were collected from plants growing natural in Mazandaran Province, region of Khashvash (36, 14', 992'' N; 52, 13', 407'' E; 2300 m above sea level), 75 km of Amol, North of Iran. The fruits were collected during the June 2011. Plant taxonomists in the Department of Biology at Urmia University, confirmed the taxonomic identification of plant species. The voucher specimens with number JCH 740 have been deposited at the herbarium of the Department of Horticulture at Urmia University.

Extraction of essential oil

Dry fruits of the plant were hydro distilled in a Clevenger type apparatus where the plant materials subjected to hydro distillation. Conditions of extraction were: 25 g of dry sample; 550 ml water, 4 h distillation. Anhydrous sodium sulphate used to remove water after extraction. Extracted oil transferred to glass flasks that were filled to the top and kept at the temperature of 4 °C in a refrigerator.

Fumigant toxicity

In order to test the toxicity, same concentrations of essential oil including 20, 27, 36, 48 and 65 µl/l air were tested on *T. castaneum* and *R. dominica*. They were applied on a filter−paper (Whatman No.1) strip measuring 4 × 5 cm that attached to the lower side of the jars lid. Twenty adults (1–7 days old) of insects were placed in small plastic tubes (3.5 cm diameter and 5 cm height) with open ends covered with cloth mesh. The tubes were hung at the geometrical center of 1 L glass jars, which then sealed with air−tight lids (HASHEMI & SAFAVI, 2012). Thus, there was no direct contact between the oil and the insects. In the control jars, oil was not applied on the filter papers. Mortality determined after 24, 48 and 72 h from commencement of the exposure. Each experiment was replicated four times for each concentration. When no leg or antennal movements observed, insect considered dead.

Data analysis

The mortality data were corrected using Abbott's formula (ABBOT, 1925) for the mortalities in the controls, and then subjected to probate analyses to estimate LC_{50} and LC_{95} values. The percentage of mortality was determined for analysis of variance (ANOVA) according to the general linear model (GLM). Significant differences identified by honest significant difference (HSD) tests at the 5% level and entered in the fig. Data processing conducted by the SPSS software version 16.0 for Windows.

Results

Yield and chemical constituent

In this research the essential oil of the fruits from *J. communis* subsp. hemisphaerica collected from region of Khashvash, gave yellowish oil with a yield of 2.31% (w/w) based on dry weights. GC-MS analyses of the fruit oil identified 14 compounds (90.58%). The main components of the oil were α−pinene (59.70%), limonene (9.66%),

myrcene (6.03%), and germacrene D (5.06%) (Table 1).

Table 1. Chemical composition of *Juniperus communis* subsp. hemisphaerica fruit oil.

No	Compound	Retention Index	Percentage
1	α-thujene	923	0.76
2	α-pinene	935	59.70
3	Sabinene	974	4.69
4	β-pinene	980	1.32
5	Myrcene	986	6.03
6	δ-2-carene	1002	-
7	α-terpinene	1017	0.27
8	Limonene	1034	9.66
9	γ-terpinene	1061	0.53
10	Terpinolene	1090	0.86
11	Terpinen-4-ol	1179	0.53
12	Z-caryophyllene	1410	–
13	E-caryophyllene	1414	1.17
14	Germacrene D	1482	5.06
	Monoterpenes		84.36
	Sesquiterpenes		6.23
	Total		90.58

Fumigant toxicity

Toxicity data indicate a remarkable difference in susceptibility between the insects (Table 2, and Fig. 1). *Tribolium castaneum* were the most resistant species to the essential oil with LC_{50} value of 107.96 µl/l air, whereas the *R. dominica* was more susceptible with LC_{50} value of 36.96 µl/l air, at a 24 h exposure time. Furthermore, with the increase of exposure time to 72 h, mortality increased and LC_{50} values decreased to 34.48 µl/l air for *T. castaneum*; and 7.94 µl/l air for *R. dominica* (Table 2). Based on the results from fumigant bioassays, the essential oil testing showed high toxicity when that was applied against insects with insecticidal activity dependent on oil concentration and exposure time. When experimental insects were fumigated for 24 h, for *R. dominica* a concentration of 48 µl/l air oil was necessary to cause mortality higher than 50%, while for *T. castaneum* concentration 65 µl/l air and 48 h exposure time was enough to cause equal mortality when were used (Fig. 1). Moreover, slopes of probit lines estimated that any increase in essential oil concentration, was imposed the high mortality to *R. dominica* (3.47 at 72 h) when compared to *T. castaneum* (3.20 at 72 h) (Table 2). Furthermore, intercept of probit line in all exposure times for *R. dominica* was higher than *T. castaneum*, showing the higher response threshold (Table 2).

Table 2. Result of probit analysis to calculate LC_{50} and LC_{95} values.

Insects	Exposure time	LC_{50}	LC_{95}	$\chi 2$ [df = 3]	*p*	Intercept	Slope
T. castaneum	24	107.96	340.95	1.94[a]	0.58	-1.69	3.29
	48	68.61	306.59	0.69 [a]	0.87	0.35	2.53
	72	34.48	112.28	1.54 [a]	0.67	0.07	3.20
R. dominica	24	36.96	181.40	0.44 [a]	0.93	1.27	2.38
	48	18.11	76.00	1.27 [a]	0.73	1.68	2.64
	72	7.94	23.62	0.81 [a]	0.84	1.88	3.47

[a] Since goodness−of−fit Chi square is not significant (P > 0.15), no heterogeneity factor is used.

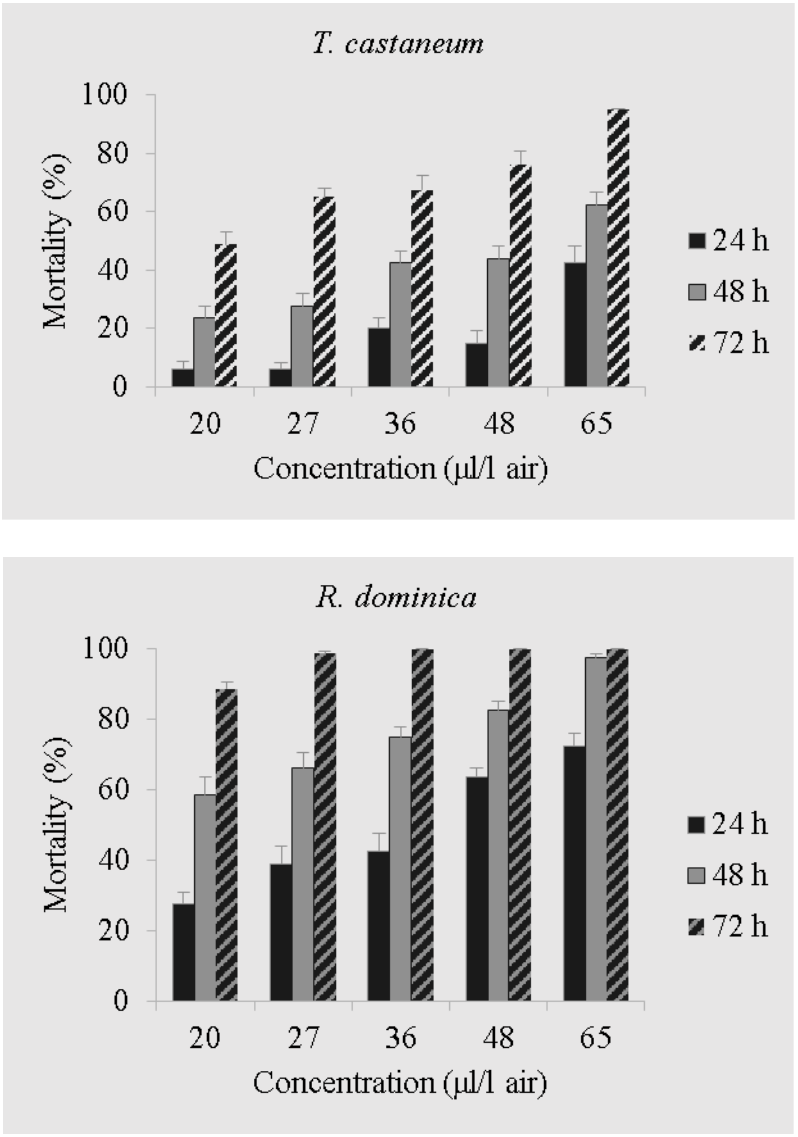

Fig. 1. Mean mortality (%) of *Rhyzopertha dominica* and *Tribolium castaneum* exposed to different concentrations of *Juniperus communis* subsp. hemisphaerica fruit oil. Different letters over columns indicate significant differences according to Tukey test at α= 0.05. Columns with the same letter are not significantly different. Vertical bars indicate standard error (±).

Discussion

The yield of essential oil from the fruits is relatively higher than other studies on *J. communis* subsp. hemisphaerica in Iran (EMAMI *et al*., 2007a, b). The fruit oil had compositions similar to those of other *J. communis* essential oils analyzed in Iran. REZVANI (2010) reported the main components of the oil that were α-pinene (46.63%), α-cedrol (12.36%), and β-pinene (4.64%). EMAMI *et al*. (2007a) also studied the composition of the *J. communis* subsp. *hemisphaerica* fruit oil. The oil contained sabinene (25.10%), α-pinene (13.60%), and limonene (9.10%) as main components. In comparison with published data, it could be clearly shown that ingredients of the essential oil of the fruits of *J. communis* subsp. hemisphaerica are similar, but with differences in their percentage depending distinctly on the region in which they are grown. Most notable differences observed in the composition of *J. communis* subsp. hemisphaerica grown in Amol (Khashvash) included the absence of α–cedrol, Δ3-carene, β-caryophyllene, and caryophyllene oxide, and the high percentage of myrcene (EMAMI *et al*., 2007a; REZVANI, 2010).

The insecticidal activity of essential oil from *J. communis* has been evaluated against

a number of insects. LANS *et al*. (2008) used the essential oil of *J. communis* L. var. depressa Pursh. to treat fleas and flies on cats and dogs in British Columbia, Canada. In another study, CHOI *et al*. (2003) tested insecticidal activity of *J. communis* oil against eggs, nymphs, and adults of *Trialeurodes vaporariorum* Westwood. In addition, essential oil of *J. communis* was evaluated for repellency against adult *Aedes aegypti* (L.), *Amblyomma americanum* (L.), *Ixodes scapularis* Say, and for toxicity against *Ae. aegypti* larvae and adults (CARROLL *et al*., 2011).

The toxicity of different essential oils used to protect against *T. castaneum* and *R. dominica* infestation has been previously studied, and these beetles have shown susceptibility to some plant−derived chemicals. Experiment has shown that, *R. dominica* is more susceptible than *T. castaneum* (Table 2 and Fig. 1). ROZMAN *et al*. (2007) studied toxicity of naturally occurring compounds of Lamiaceae and Lauraceae against *Sitophilus oryzae* (L.), *R. dominica* and *T. castaneum*. They observed that *R. dominica* was more susceptible than *T. castaneum.* EBADOLLAHI (2011) evaluated toxicity of essential oil of *Agastache foeniculum* [Pursh] Kuntze against *R. dominica* and *T. castaneum.* Results showed that, *R. dominica* (LC_{50}= 14.17 µl/l) was more susceptible than *T. castaneum* (LC_{50}= 22.24 µl/l), at 24 h exposure time. LEE *et al*. (2004) tested the fumigant toxicity of six essential oils and 1, 8−cineole against *S. oryzae*, *T. castaneum*, and *R. dominica*. In that experiment, *R. dominica* was found more susceptible than the other species. HOSSEINI *et al*. (2013) reported fumigant toxicity of essential oil from *Salvia leriifolia* (Benth.) against *Sitophilus granarius* (L.) and *R. dominica*. LC_{50} values at 24 h were estimated 79.17 µl/l air for *S. granarius*, and 25.87 µl/l air for *R. dominica*.

GC−MS analyses of the oil revealed that the percentage of monoterpenoids was higher than the other compounds (Table 1). The insecticidal constituents of many plant extracts and essential oils are monoterpenoids. Due to their high volatility, they have fumigant action that might be of great importance for stored product insects (LEE *et al*., 2002, 2004; HASHEMI & SAFAVI, 2012; HOSSEINI *et al*., 2013). The α−pinene is one of these monoterpenoids. It is characterized as the main component (59.70%) of the fruits of *J. communis* subsp. hemisphaerica essential oil. There are numerous reports on toxicity of the α-pinene to our experimental insects. LEE *et al*. (2002) reported toxicity of α−pinene to *T. castaneum*. The oils extracted from leaves and the fruits of *Platycladus orientalis* (L.) Franco containing *a*−pinene as a major component (35.2%, 50.7%), respectively, was found to be the most effective against *T. castaneum* (HASHEMI & SAFAVI, 2012). α−pinene as a major compound (15.89%) of *S. leriifolia* was toxic on *R. dominica* (HOSSEINI *et al*., 2013).

This study indicates that essential oil of *J. communis* subsp. hemisphaerica is a source of biologically active vapor which may potentially prove to be efficient insecticide. Toxicity screening of essential oil showed significant activity against *T. castaneum* and especially *R. dominica*. This study will provide a basis in the future work with *J. communis* subsp. hemisphaerica particularly from Iran.

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Recognition of Endemic Plants in Zagros Region (Case Study: Lorestan Province, Iran)

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Abstract. The present study was carried out in the Hashtadpahlou and Sefidkoh mountains which are important vegetation regions in the western Iran, Lorestan. The endemic plants and their life form in these mountain regions were determined. From the view point of regional elements, plants of these regions belong to Irano-Turanian region. Totally, 86 endemic plants from 18 families were recognized. The results show that the important families are Asteraceae (20 species), Fabaceae (14 species), Lamiaceae (13 species). The highest number of species investigated for *Astragalus* genus (11 species). Life forms of the plant species of region include: geophytes 26.44%, therophytes 16.09%, hemicryptophytes 18.38%, chamaephytes 31.04%, phanerophytes 8.08% .The high frequency of chamaephytes and geophytes can be attributed to high altitude and cold climate. Generally, it can be declared that the habitats of these ecosystems have high diversity due to the presence of 87 endemic plants and this pattern resulted from climate conditions and geomorphology of territory.

Keywords: Endemic plants, Life form, Irano-Turanian, Zagros, Iran.

Introduction

Biological variation of plant species are important in plant ecological studies (MAHMOUDI, 2007) and what enhanced today the increasing importance of biological diversity is its role in maintaining the stability of ecosystems (ESMAEILZADEH & HOSSEINI, 2006). The composition of plant and animal in a given region is important for natural resources management (AKSOY & UZUN, 2011) and more accurate recognition plant and animal species provides suitable approach for better protection of ecosystems. Overpopulation, pollution, soil erosion, forest harvesting operation and other incorrect utilization of natural resources have caused many problems in the environment. To preserve these resources, one essentially uses the plants properly and to achieve this important matter, one should be aware of flora and its relationship with the environment. Endemic species are those plants that distribution is restricted only to a particular region (ANDERSON, 1994; BULUT & YILMAZ, 2010). In recent years, awareness of the importance and role in relation to endemic species in conservation planning has been rising (SLATYER, 2007). In general, studies of these plants are useful for :1) knowing vegetation in the past; 2) determining the relationship of taxonomy; 3) identifying floristic area; 4) determining the optimum planning the protected parts, and 5) prioritizing strategies for protection (DHAR, 2002). Each plant has the unique ecological range and can tolerate a certain rate of variation of environmental conditions (ZAREI & ASSADI, 2008).

Given the importance of plants in environmental studies, identifying vegetation cover of different areas could play significantly role in planning a variety of programs particularly in the preservation, restoration, management and identification of plant species, including endemic plants. Floristic studies in Iran for conservation of natural resources, biodiversity and making the management planning are concerned. Iran has one of the most attractive rich flora in the South - Western Asia, and this is because of the large area, diversity of climate and topography (GHAREMANINEJAD, 2010). Endemic plants are not few in Iran, so their study plays a significant role in the preservation of natural resources. In this regard, recognition of endemic plant species was carried out in Zagros zone, Western Iran.

Materials and Methods

Study site

To study the endemic plant in this region, the Hashtadpahlu and Sefidkoh mountains were selected. The Hashtadpahlu range (ABRARI & VEISKARAMI, 2001) is located in south-western of Khorramabd (Lorestan province) and the northern aspect of this mountain (3000 ha) was studied (latitude: 33° 13′, 33° 16′; longitude: 48° 23′, 48° 27′). Minimum and maximum elevations are 1000 and 2900 m above sea level, respectively. Another part of the study area is the Sefidkoh Mountains which positioned in the north-west of Khorramabad in Lorestan, including 1100km^2 area(latitude:48° 20′,47° 50′; longtitude:33° 30′,33° 45′) with elevation of 950-3060 m above sea level (VEISKARAMI, 2000). Annual mean of precipitation is about 400-500 mm and soil types belong to Entisols and Inceptisols orders (VEISKARAMI, 2000).

Data collection

In order to identify the flora in these regions, the collecting samples of all the existing plants was conducted in the growing season. To investigate plant species, the scientific resources of flora were used (GHAHREMAN, 1996; DAVIS, 1988; RECHINGER, 1998). The life form of plants was identified using the biological classification system of Raunkier (GHAHREMAN, 1996).

Results

Based on the study of vegetation, 86 endemic species belonging to 54 genera and 20 plant families were identified (Table 1). The results showed that the highest number of species belonged to Asteraceae (16 species), Fabaceae (14 species), Lamiaceae (13 species), in such way that these species consisted the 54.01% of total plants (Table 2). 72 perennial species (83.72%) and 14 annual species (16.28%) were identified which belonging to 19 families of dicotyledonous and 1 family of monocots (Table 3). *Astragalus* genus (Fabaceae family) had the highest number of species (11 species) in these regions (Table 1). In terms of the life form of plants, it was determined that 23 species of geophytes (44/26%), 14 species of therophytes (09/16%), 16 species of hemicryptophytes (39/18%), 27 species of chamaephytes (04/31%), 7 species of phanerophytes (05/8%) were presented in these areas (Fig. 1).

Table 1. List of endemic plants in mountains regions, Zagros, Western Iran

Family	Taxa	Life form	Duration
ARISTOLOCHIACEAE	*Aristolochia olivieri* COLLEGNO	Ge	P
BORAGINACEAE	*Lappula barbata* (M.B.) GURKE	Th	A
	Lappula sinaica (DC.) ASCHERSON ex SCHWEINF	Th	A
	Lappula spinocarpus (FORSSK.) ASCHERSON & O. KUNTZE	Th	A
	Myosotis Koelzii H.RIEDL.	Th	A
	Nonnea suchtelenioides H. RIEDL	Hem	P

	Onosma kotschyi BOISS.	Hem	P
	Onosma platyphyllum H. RIEDL	Hem	P
CARYOPHYLLACEAE	*Dianthus orientalis* ADAMS. ssp. *scoparius*	Ch	P
	Gypsophila persica BARKOUDAH.	Th	A
	Silene pseudaucheriana MELZH.	Ch	P
ASTERACEAE	*Anthemis cretica* L.	Ge	P
	Centaurea geluensis BOISS. & HAUSSKN.	Hem	P
	Centaurea koeieana BORNM	Hem	P
	Cephalorrhyncus rechingerianus TUISL.	Ge	P
	Cirsium spectabile DC.	Hem	P
	Cirsium bracteosum DC.	Hem	P
	Cousinia disfulensis BORM.	Hem	P
	Cousinia haussknechtii WINKL.	Hem	P
	Cousinia khorramabadensis Bornm.	Hem	P
	Echinops endotrichus RECH. f.	Hem	P
	Iranecio paucilobus (DC.)B.Nord.	Hem	P
	Helichrysum oligocephalum DC.	Hem	P
	Phagnalon persicum BOISS.	Hem	P
	Postia puberula BOISS. & HAUSSKN.	Ch	P
	Scorzonera calyculata BOISS.	Hem	P
ASTERACEAE	*Senecio pseudo-orientalis*	Hem	P
CRASSULACEAE	*Rosularia elymaitica* BOISS. & HAUSSKN.) BERGER	Hem	P
	Umblicus intermedius BOISS.	Ge	P
	Umblicus trapaeolifoius BOISS.	Ge	P
CRUCIFERAE	*Graellsia saxifragifolia* (DC.) BOISS. ssp *saxifragifolia*	Ch	P
	Hesperis odorata DVORAK.	Ch	P
	Hesperis kurdica DVORAK et HADAC.	Ch	P
	Physorhyncus chamaerapistrum BOISS.	Ch	P
	Sameraria stylophora (JAUB. & SPACH.) BOISS.	Th	P
CUSCUTACEAE	*Cuscuta kotschyana* BOISS.	Th	A
EUPHORBIACEAE	*Euphorbia craspedia* BOISS.	Th	A
FAGACEAE	*Quercus brantii* LINDL. var. *persica*	Ph	P
FUMARIACEAE	*Corydalis verticillaris* DC.	Ge	P
LAMIACEAE	*Cyclotrichum strausii* (BORNM.) RECH. f.	Ch	P
	Nepta humilis BENTH.	Th	A
	kotschyi BOISS *Nepta*	Ge	P
	Nepta ptraea BENTH.	Th	A
	Nepta strausii HAUSSKN. & BORNM	Th	A
	Phlomis anisodonta BOISS.	Ge	P
	Phlomis olivieri BENTH.	Ge	P
	Salvia reuterana BOISS.	Ge	P

	Salvia sclreolepis BERNM. ex HED	Ge	P
LAMIACEAE	*Scutellaria nepetifolia* BENTH.	Ch	P
	Stachys benthamiana BOISS.	Ch	P
	Stachys melampiroides HAND – MTZ.	Th	A
	Stachys perspolitana BOISS.	Th	A
FABACEAE	*Astragalus ochinops* Boiss.	Ch	P
	Astragalus baba- alliar PARSA	Ph	P
	Astragalus babakhanloui MASSOUMI & PODL.	Ch	P
	Astragalus bodeanus FISCHER	Ch	Ch
	Astragalus veiskarami Zarre,podlech& Sabatii.	Ch	P
	Astragalus ecbatanus BUNGE	Ch	P
	Astragalus galbineus MAASSOUMI	Ch	P
	Astragalus gaubae BORNM	Ch	P
	Astragalus ibicinus BOISS. & HAUSSKN	Ch	P
	Astragalus leonardii MAASSOUMI	Ch	P
	Astragalus ptychophyllus BOISS.	Ch	P
	Onobrychis melanotricha BOISS.	Ch	P
	L. *Ononis spinosa*	Ch	P
	Vicia kotschyana Boiss.	Ge	P
LILIACEAE	*Allium haemanthoide*s BOISS. & REUT. & REGEL	Ge	P
	*Allium laev*e WENDELBO & VON BOTHMER	Ge	P
	Allium jesdianum BOISS&BUSHE.	Ge	P
	Nectroscurdum koelzii WENDELBO.	Ge	P
PLUMBAGINACEAE	*Acantholimon brachystachys* BOISS.	Ch	P
	Acantholimon bromifolium BOISS. var. bromifolium	Ch	P
	Acantholimon eshkerensis BOISS. & HAUSSKN	Ch	P
PRIMULACEAE	*Dionysia haussknechtii* BORNM. & STRAUSS.	Ch	P
ROSACEAE	Amygdalus haussknechtii (C. K .SCHNEIDER) BORNM	Ph	P
	Amygdalus lycioides SPACH.	Ph	P
	Cerasus brachypetelum BOISS.	Ph	P
	Cerasus microcarpa (C. A. MEY) BOISS.	Ph	P
RUBIACEAE	*Galium ceratocarpon* BOISS.	Ch	P
SCRUPHLARIACEA	*Scrophularia nervosa* BENTH. ssp. *nervosa*	Ge	P
UMBELLIFERAE	*Beuplorum haussknechtii* BOISS.	Th	A
	Bunium luristanicum RECH. f	Ge	P
	Bunium rectangulatum BOISS. & HAUSSKN	Ge	P
	Ferula macrocolea (BOISS.) BOISS	Hem	P

Ph – phanerophyte; Th – therophyte; Ch – chamephyte; Hem – hemicryptophyte; Ge – geophyte; A – annual; P - perennial

Table 2. List of families and number of species in the studied regio

Family	No.Species	No.Species(%)
ARISTOLOCHIACEAE	1	1
BORAGINACEAE	7	8/14
CARYOPHYLLACEAE	3	3/45
ASTERACEAE	16	32/98
CRASSULACEAE	3	3/45
CRUCIFERAE	5	5/75
CUSCUTACEAE	1	1/15
EUPHORBIACEAE	1	1/15
FUMARIACEAE	1	1/15
FAGACEAE	1	1/15
LAMIACEAE	13	14/94
FABACEAE	14	16/09
LILIACEAE	4	4/59
PLUMBAGINACEAE	3	3/45
PRIMULACEAE	2	2/29
RANUNCULACEAE	1	1/15
ROSACEAE	4	4/59
RUBIACEAE	1	2/29
SCROPHOLARIACEA	1	1/15
UMBELLIFERAE	4	4/59

Table 3. Family, Genus and taxa in each plant group

Plant Group	Family	Genus	Taxa
Pteriphytes	0	0	0
Monocotyledon	1	2	4
Dicotyledon	19	52	82
Total	20	54	86

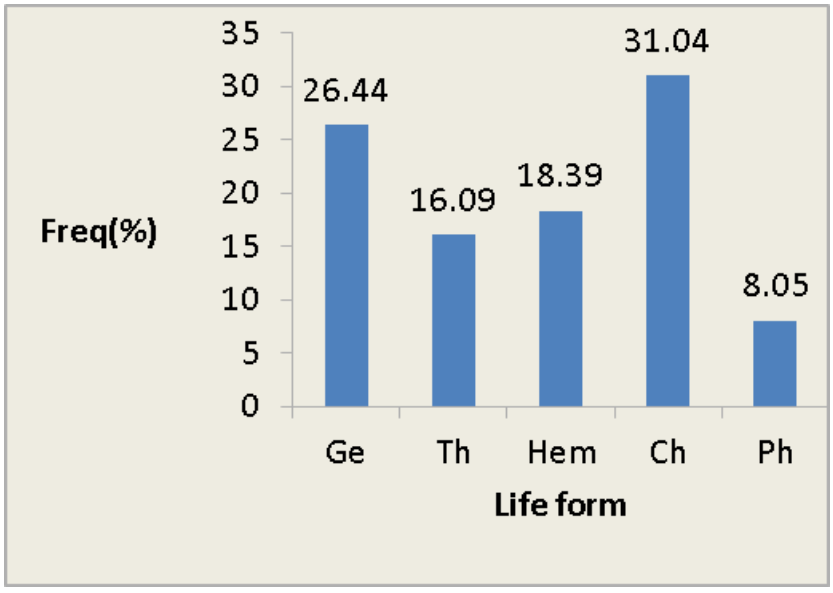

Fig. 1. Frequency of life form of endemic plants in region

Discussion

Identification of vegetation in given region and ecological analysis of their nature can help to detect the ecological characteristics and growth potential of the region (ESMAEILZADEH & HOSSEINI, 2006). Presence of 87 endemic species in these mountains is indicator for high plant biodiversity in these areas. The diversity in life form of plants of this region is the indication of adaptation to climatic and soil conditions. Life form of plants show taxonomic features of them and also indicates their adaptation to environmental conditions (ASRI, 2008).The high frequency of chamephytes and geophytes may be attributed to the high elevation and cold region that is consistent with SAFIKHANI *et al*. (2002) research. The high prevalence of chamephytes life form shows that agriculture operation is few in these zones. Chamephytes species, in the form of cushion and thorn, adapted to drought, high light conditions and winds (MEMARIANI *et al*., 2009).

The presence of hemicryptophytes is the sign of mountain areas. However, the lowest frequency of life form belong to phanerophytes , but this reflects suitable ecological conditions for the establishment of phanerophytes. The presence of *Astragalus* genus with 11 species reveal highland condition which is in agreement with ASSADI study (2009).The existence of species belonged to *Astragalus* genus such as *Astragalus anacardius* BUNGE., *Astragalus baba-alliar* PARSA, *Astragalus bodeanus* FISCHER and *Astragalus galbineus* MAASSOUMI is result of suitable condition in the Zagros region. Genus of *Astragalus* includes plants which produce gum tragacanth and so classified as medicinal plant (MAHMOUDI *et al*., 2009). The main feature of Irano-Touranian vegetation is the presence of species such as *Astragalus* and *Cousinia* genera (ASRI, 2008), and these two genera present within this region. The main reason for the dominance of Irano - Turanian vegetation in this region may be due to the remoteness of the area from other areas and particular environmental conditions (PAIRANJ *et al*., 2011). The presence of 86 endemic plants reflects the fact that there is high biodiversity within these habitats and this is due to climatic and geomorphology condition of their territory. The mountain areas due to the special geographical position and various environmental situations are rich regarding the existence of endemic plant species (IUSZ *et al*., 2011). High frequency of hemicryptophytes is clearly a sign of orophilous vegetation of endemic plants within these mountain ranges of Zagros region. MELENDO *et al*. (2003) stated that the frequencies of the hemicryptophytes in the Mediterranian region have been linked to increased rainfall and reduced heat.

The presence of therophytes plants can be an effective strategy to prevent water shortage due to reduced water and humidity (DA COSTA *et al*., 2007).In areas with winter rains, therophytes are more resistant to summer drought than hemicryptophytes and geophytes because the therophytes appear as the seeds but the hemicryptophytes and geophytes form vegetative organs in the summer (VAN DER MERWE & VAN ROOYEN, 2011).Since endemic species in these areas belong to Irano-Turanian vegetation elements ,so it can be stated that these areas(Hashtadpahlu and Sefidkaoh mountains) belong to the Irano-Turanian region of Iran. On the basis of the high number of endemic plants (86 species) in this region with small area, it should be declared that these habitats are a genetic reservoir for many species and it is necessary to protect these sites.

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The Comparison of the Winter Diet of Long-Eared Owl Asio otus in Two Communal Roosts in Lublin Region (Eastern Poland) According to Selected Weather Conditions

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Abstract. The survey was conducted in two test areas in Wólka Kątna and Zemborzyce in Eastern Poland in winter 2012/2013. The winter diet of Long-eared Owl *Asio otus* in the test areas differed significantly. In Zemborzyce the Levins food niche breadth index and the Wiener-Shannon biodiversity index were strongly correlated with the average temperature and the snow depth, and not correlated with the precipitation. In Wólka Kątna no correlation was found. No correlation between the weather factors and the number of each prey species was found, except the Tundra Vole *Microtus oeconomus* in Zemborzyce, which occurrence in owls' pellets was positively correlated with the temperature and negatively correlated with the snow depth. Seven factors describing the owls' diet was chosen: average number of prey in one pellet, average number of prey per bird per day, share of *Arvicolidae* and *Muridae* in prey number and prey biomass, and the biomass of prey per bird per day. The share of *Arvicolidae* in biomass negatively correlated with the precipitation on the Zemborzyce test area and no other dependency between diet factors and weather conditions was found.

Key words: Long-eared Owl, winter diet, *Asio otus*, weather

Introduction

The Long-eared Owl *Asio otus* is the second numerous owl in Poland (SIKORA *et al*., 2007). The fact of forming communal roosts in winter, where many birds rest together, makes the species one of the best known in aspect of diet during non-breeding season (HOLT, 1997; ŻMIHORSKI, 2005; BIRRER, 2009; DZIEMIAN *et al*., 2012; KITOWSKI, 2013). Fewer papers treat about the effect of weather factors on the birds' activity and the food composition in different types of habitat (CANOVA, 1989; RUBOLINI *et al*., 2003; ROMANOWSKI & ŻMIHORSK,I 2008). In case of more frequent weather anomalies, the knowledge of their impact on birds may be important in understanding their ecology and for better protection (KUNDZEWICZ & JUDA-REZLER, 2010).

The aim of present study was to survey and compare the effect of selected weather conditions on the diet of Long-eared Owl on two test areas in Eastern Poland.

Material and Methods

Pellets of Long-eared Owl were collected from two communal roosts in Lublin Region, Eastern Poland – Wólka Kątna (51˚10'56 N, 22˚32'16" E) and Zemborzyce (51˚25'35" N, 22˚16'49" E) (Fig 1). In both cases, communal roosts were

located on pines and the pellets were gathered from under those trees at intervals of 6 to 19 days. Habitat in which birds were roosting differed between the test areas. In Wólka Kątna, the roosting trees were located on the edge of the small forest patch surrounded by agricultural landscape. The area of the patch was 0.3 km², and the distance to the field, where birds could hunt was less than 50 meters. In Zemborzyce, the communal roost was also placed in the middle of the forest edge, between the forest and the artificial lake. The area of the forest was 13.7 km², and the distance to the open space suitable for birds to hunt was over 1000 meters.

Fig. 1. Test areas in Eastern Poland: 1 – Wólka Kątna, 2 – Zemborzyce.

In Wólka Kątna the pellets were collected 10 times, in Zemborzyce 9 times during the period of the survey. The pellets were prepared for analysis by standard methods (RUPRECHT *et al.*, 1998). The number of vertebrate prey species was determined on the basis of skulls, mandibles, teeth, and bone remains (PUCEK, 1984; KITOWSKI *et al.*, 2005). Due to the high degree of fragmentation of some remains, preventing species identification, some prey was grouped, like *Microtus, Apodemus* etc. (Tab. 1, Tab. 2). The prey biomass was estimated as in literature (KITOWSKI, 2013).

The weather data was received from the Institute of Meteorology and Water Management (IMGW), from meteorological stations in Radawiec near Lublin and from Puławy (IMGW, 2013). For analysis, the data of daily precipitation, daily average temperature and snow cover were taken. Each part of data was averaged for the period between subsequent pellet gatherings.

The analyzes included correlations between weather conditions and average number of prey in one pellet, prey number and biomass per bird per day, and the share of *Arvicolidae* and *Muridae* in prey number and biomass during each interval. The correlation between each prey taxon and the weather conditions was also analyzed.

The width of the food niches of owls was estimated using the formula by LEVINS (1968):

$$B = 1/\Sigma\, pi^2,$$

where pi is the proportion of the prey category *i* in the total biomass of the owl's diet.

Shannon-Wiener biodiversity (H) indices were calculated for trophic diversity at species levels using the next formula (KREBS, 1994):

$$H = -\Sigma\, [Pi \log (Pi)].$$

Both indexes were analysed to check the correlation with weather conditions.

To compare the test areas, prey was grouped into two families – *Arvicolidae* and *Muridae*, and the "*Other*" group for avian prey, including also reptile and insects, both found once in the survey. The 2×2 Chi-square test was used for *Arvicolidae* and for *Muridae* in number and biomass of prey, to compare the communal roosts (Tab.1). The *Other* group wasn't compared, due to the low number of prey.

All statistical analysis was conducted at significance level $\alpha = 0.05$. Spearman's correlation was used to examine the dependence of each factor, and Mann-Whitney U test was used to compare the data from different test areas.

Results

In total, 237 prey items were selected from pellets in Zemborzyce test area and 461 in Wólka Kątna. The owls' diet differed significantly between the test areas (Tab. 1).

In almost every control on both gathering places, the Common vole *Microtus arvalis* was the most numerous prey species. In two cases, in pellets collected on the Zemborzyce test area, the most numerous taxon was Tundra vole *Microtus oeconomus*. Other taxa occurred in the lower number and had a lower share in the biomass (Tab. 1).

Table 1. The winter food composition of Long-eared Owl on communal roosts in Wólka Kątna and Zemborzyce in Eastern Poland

	Species	%N		%B	
		Wólka Kątna	**Zemborzyce**	**Wólka Kątna**	**Zemborzyce**
Arvicolidae	*Microtus arvalis*	55.70%	66.59%	51.03%	65.45%
	Microtus oeconomus	22.36%	3.04%	28.04%	4.08%
	Microtus subterraneus	1.27%	2.60%	1.04%	2.29%
	Clethrionomys glareolus	1.27%	5.21%	1.04%	4.58%
	Microtus spp.	2.95%	3.25%	3.28%	3.87%
Muridae	*Apodemus*	6.75%	9.98%	7.33%	11.61%
	Apodemus agrarius	0.42%	0.43%	0.35%	0.38%
	Sylvaemus	0.84%	1.52%	0.83%	1.61%
	Muridae spp	1.27%	4.34%	1.10%	4.04%
	Mus musculus	0.42%	0.65%	0.32%	0.52%
	Micromys minutus	2.11%	1.74%	0.81%	0.72%
Other	*Aves*	3.80%	0.65%	4.58%	0.84%
	Insecta	0.42%	0.00%	0.02%	0.00%
	Reptilia	0.42%	0.00%	0.24%	0.00%
Chi-square test	*Arvicolidae*	$\chi^2 = 0.67$	p = 0.413	$\chi^2 = 36.13$	p<0.0001
	Muridae	$\chi^2 = 5.03$,	P = 0.025	$\chi^2 = 155.03$	p<0.0001

Table 2 The correlation between the Levin's index and the Wiener-Shannon index, and selected weather factors on test areas in Wólka Kątna (W) and Zemborzyce (Z) in Eastern Poland (Spearman test).

Spearman correlation			temperature (˚C)		precipitation (mm)		snow cover (cm)	
			R	p	R	p	R	p
Levin's index	W	2.40 (SD = 0.89)	-0.310	0.384	0.055	0.881	0.411	0.237
	Z	2.61 (SD = 0.79)	**0.850**	**0.003**	0.100	0.798	**-0.683**	**0.042**
Wiener-Shannon index	W	1.11 (SD = 0.32)	-0.248	0.489	-0.006	0.987	0.251	0.483
	Z	1.17 (SD = 0.31)	**0.817**	**0.007**	0.067	0.864	**-0.750**	**0.020**

The occurrence of individual taxa on both test areas did not correlate with selected weather conditions, except the Tundra vole, which occurrence in pellets from Zemborzyce test area was positively correlated with the temperature (Spearman test, R = 0.729, p = 0.026) and negatively correlated with the snow cover (Spearman test, R = -0.763, p = 0.017). The Wiener-Shannon H index and the Levin's B index

were count for each interval on both test areas. There was no significant difference in these factors between the test areas (Mann-Whitney U test, U = 35, p = 0.438 for the Levin's index and U = 38, p = 0.596 for the Wiener-Shannon index). In Wólka Kątna none of the indexes were correlated with the weather factors. In Zemborzyce the food niche breadth and the biodiversity index were strongly, positively correlated with the temperature and negatively with the snow cover, which is presented in Table 2.

On the communal roost in Zemborzyce, the only factor, which was correlated with the weather conditions (R=-0.667, p=0.050), was the share of *Arvicolidae* in the total prey biomass. Other factors were not correlated with weather. In Wólka Kątna none of factors studied was correlated with weather. The selected weather conditions did not also influence on the functioning of the owls' digestive system, which is illustrated by lack of differences in the number of prey individuals per pellet (Table 3).

Table 3. The dependency of Long-eared Owls' diet factors on the selected weather conditions on test areas in Eastern Poland (W – Wólka Kątna, Z – Zemborzyce)

		temperature (˚C)		precipitation (mm)		snow cover (cm)	
	Test area	R	p	R	p	R	p
Number of prey in pellet	Z	-0.238	0.570	0.476	0.233	0.048	0.911
	W	0.200	0.606	-0.050	0.898	-0.239	0.444
Number of prey/day/individual	Z	0.042	0.915	0.435	0.242	-0.050	0.898
	W	0.115	0.751	-0.539	0.107	-0.460	0.181
%N *Arvicolidae*	Z	-0.133	0.732	-0.500	0.170	0.017	0.966
	W	0.006	0.987	-0.333	0.347	-0.325	0.359
%B *Arvicolidae*	Z	-0.100	0.798	**-0.667**	**0.050**	-0.017	0.966
	W	0.091	0.803	-0.103	0.777	-0.337	0.340
%N *Muridae*	Z	0.250	0.516	0.250	0.516	0.067	0.864
	W	-0.006	0.987	0.333	0.347	0.325	0.359
%B *Muridae*	Z	0.250	0.516	0.250	0.516	0.067	0.732
	W	0.006	0.987	0.030	0.934	0.264	0.461
Biomass/day (g)	Z	0.133	0.732	0.350	0.355	-0.283	0.460
	W	0.139	0.701	-0.564	0.090	-0.472	0.168

Discussion

The Long-eared Owl is mentioned as a typical feeding-specialist, mostly hunting small rodents, especially voles *Arvicolidae* (KITOWSKI *et al.*, 2005; ROMANOWSKI & ŻMIHORSKI, 2008). The present study corresponds with these results and is similar to other data from Eastern Poland (DZIEMIAN *et al.*, 2012; STASIAK *et al*,. 2012; KITOWSKI, 2013).

Analyzing the Levin's B index and the Shannon-Wiener H index we obtained the values similar to the data from other test areas in Eastern Poland. The values of the niche breadth obtained in Wólka Kątna (1.64 to 4.20), and in Zemborzyce (1.43 to 3.95), was corresponding to the other results which were comprised between 1.10 and 5.87 (KITOWSKI *et al.*, 2005; ŻMIHORSKI, 2005; DZIEMIAN *et al.*, 2012; STASIAK *et al.*, 2012; KITOWSKI, 2013). The biodiversity H index was also corresponding with other data from the area of the research (STASIAK *et al.*, 2012).

Like in other surveys, the majority of the birds' prey were species connected with large open areas like fields and meadows (*Microtus arvalis, M. oeconomus,* etc.), while forest species (e.g. *Myodes glareolus*) were occasionally hunted (WIJNANDTS, 1984; BIRRER, 2009). The low number of forest species could not be expected in the Zemborzyce test area, because of the long distance from the owls' communal roost to

the nearest open space. Birds used for hunting rather the distant open space, than the forest nearby. Earlier survey on correlation between Long-eared Owls' diet and weather conditions, conducted on Zemborzyce test area (which is being used by the species as a communal roost each year for a long time), is less accurate than present study, because of only four pellet gatherings in two years of the research. The relationship between the owls' diet and the weather conditions shown in mentioned study (WIĄCEK *et al.,* 2011), was confirmed in this paper, as the Wiener-Shannon and Levin's indexes did differ according to the temperature and the snow cover. Also in Northern Italy, the temperature did affect on the food niche breadth index, while no correlation between birds' diet and rainfall was found (RUBOLINI *et al.,* 2003). The opposite to presented results can be found in the survey conducted in Central Poland, where the Levin's index differed according to the precipitation factor only (ROMANOWSKI & ŻMIHORSKI, 2008), in our survey such correlation wasn't found. In opposite, in Wólka Kątna, the niche breadth and the biodiversity index didn't differ in aspect of weather conditions, which can be explained by the fact of easy access to different landscapes – fields, meadows, forest patch, human settlements and the ecotone.

The snow cover is mentioned as an important weather aspect in the literature. This is because of the owls' specialization to catching they prey on the ground. Some small mammals, especially voles move rather under the snow cover, which makes them more difficult to hunt in case of the cover's larger thickness (CANOVA, 1989; GALEOTTI & CANOVA, 1994; DZIEMIAN *et al.,* 2012). *Muridae* are being found rather over the snow, which usually causes the increase of their participation in the number and the biomass of the birds' prey, while the share of *Arvicolidae* decreases (ROMANOWSKI & ŻMIHORSKI, 2008; DZIEMIAN *et al.,* 2012). In presented study this didn't take place, as none of the diet factors (share of both main prey groups) was dependant on weather. Also the increase of the share of birds in owls' diet, noted in other studies (CANOVA, 1989), has not been observed in this survey, while birds were occasional prey on both surveyed test areas. In presented research the precipitation factor meant also rainfall and snowfall, and on the Zemborzyce test area this factor was strongly, negatively correlated with the share of *Arvicolidae* in prey biomass. The survey conducted in Western Poland, this factor affected the food niche breadth, but not the share of any species or group of species.

The small amount of Long-eared owls' diet factors correlated with selected weather conditions suggests the high stability of the birds' diet in the area of the research, but lower in the forest habitat. The differences between the test areas mean the species can adapt to the different habitat, and maintain the diet stable. The specialization in feeding or in habitat preferences can provide the species to be more exposed to habitat changes. In case of other owl species, the high degree of association with the specific habitat factors can provide birds to disappearance from the previously held area (KITOWSKI & STASIAK, 2013). This fact should provide to further research of this aspect of the owls' diet.

Conclusions

1. The diet of Long-eared Owl in two surveyed test areas differed significantly.

2. On Zemborzyce test area the food niche breadth and the biodiversity index were correlated with the temperature and the snow depth, while in Wólka Kątna no such correlation was found.

3. The only diet factor correlated with the weather was the number of *Arvicolidae* on Zemborzyce test area.

4. The diet of Long-eared Owl in Eastern Poland was stable in the time of the survey.

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Synopsis

Croatian Experience in Waste Oil Management

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Abstract. This paper describes the course of introducing and implementing regulations ensuring efficient waste oil (lubricant and edible oil) management, as well as results obtained based on long-term experience in monitoring and reporting on the management of this special waste category. According to the results from 2007 to 2012, between 6 000 and 7 000 t of waste lubricant oil was collected in the Republic of Croatia. Quantities of collected waste edible oil in the same period indicate low recovered amounts compared to the estimated annual quantities of fresh edible oil placed on the market (50 000 t), i.e. supposed quantities of waste edible oil recovered vary, from 3.6 % to 8.6%.

Key words: Waste edible oil; Waste management; Waste oils; Waste oil management

Introduction

According to the European Commission Eurostat office of statistics (EUROPEAN COMMISSION, 2013), in 2010 the total quantity of waste produced from all economic activities and households at the EU-27 amounted to 2 502 million tonnes. In the same time the quantity of processed waste (including the waste imported into the EU) was 2 336 million tonnes. Almost half of the mentioned amount (45.4 %) of waist, processed within the EU, was treated by the means of disposal, primarily at the disposal sites, part was disposed of in proximity or within the mines, and remained was released into the water. 49 % of the total waste quantity processed in the EU in 2010 was recycled using procedures of extracting valuable components, while remaining 5.6 % was processed by means of incineration with or without using thermal energy.

Mentioned waste quantities, as well as activities related to its processing and/or treatment, indicate the significance of waste management, which has an emphasized position in environment protection strategies in all European countries. Developed countries focus on minimizing and recycling waste, thus contributing to closing of the circle of raw material use by creating materials from waste as an input for new production. Consideration of life cycle has lately been increasingly introduced as the leading principle of resource management. The environmental impacts are being studied throughout a product's life span in order to wherever possible avoid or minimize transfer of environmental problems from one stage of life span to

another or transfer from one country to another via market instruments.

An established and efficient waste management system is an important component of the contemporary society. In order to ensure its further development and better efficiency economic incentives are required to ensure reduction of waste quantities at the place of occurrence, separate collection, processing and recycling.

Croatia's legislative system regulates waste management in large part, although there are areas that are not harmonized with EU requirements and standards. However, evasion of implementation presents the biggest challenge. Even though activities and measures in the field of waste management contributing to fulfilling set strategic goals have been implemented in Croatia, some segment still do not demonstrate the expected dynamics or experience difficulties in implementation. Thus, further strengthening of the capacities in the terms of authorities for waste management is essential, as well as the improvement in private sector participating in the waste management process as well as development of new and enhancement of existing economic instruments, such as already introduced fees for recycling and collection of special categories of waste and fees for pollution by waste disposal. Further efforts are also needed in the education of experts for pollution by waste disposal, who will work on this problem.

Within the Croatian legislative framework, a special place belongs to the management of special categories of waste, that is regulated by a series of individual ordinances (Ordinance on packaging and packaging waste, Official Gazette of the Republic Croatia, OG 97/05, 115/05, 81/08, 31/09, 156/09, 38/10, 10/11, 81/11, 126/11, 38/13, 86/13; Ordinance on waste tyre management, OG 40/06, 31/09, 156/09, 111/11, 86/13; Ordinance on waste oil management, OG 124/06, 121/08, 31/09, 156/09, 91/11, 45/12, 86/13; Ordinance on waste batteries and accumulators management, OG 133/06, 31/09, 156/09, 45/12, 86/13; Ordinance on the management of end-of-life vehicles, OG 136/06, 31/09, 156/09, 53/12, 91/13; Ordinance on the method and procedures for managing waste containing asbestos, OG 42/07; Ordinance on medical waste management OG 72/07; Ordinance on the management of waste electrical and electronic appliances and equipment, OG 74/07, 133/08, 31/09, 156/09, 143/12, 86/13; Ordinance on construction waste management, OG 38/08; Ordinance on management of wastewater treatment sludge when used in agriculture, OG 38/08; Ordinance on management of waste from the titanium dioxide industry, OG 70/08; Ordinance on the management of polychlorinated biphenyl and polychlorinated terphenyls, OG No. 105/08). Among other matters these ordinances prescribe development of systems of separate collection and recycling of individual special categories of waste (OG 94/13), including: bio waste, waste textile and footwear, waste packaging, waste tyres, waste oils, waste batteries, waste vehicles, waste consisting asbestos, medicinal waste, waste electric and electronic devices and equipment, waste naval vehicles, sea waste, construction waste, waste sludge from the waste water purification plants, waste from the production of titan oxides, waste poly chloride biphenyl and poly chloride terphenyl.

Of the relatively high number of special categories of waste, Croatia as well as some other European countries started to organized management of categories of waste oils. Hence, this paper will demonstrate some of our experiences in that field.

How it all started

Mid 1970s are considered as the beginning of the introduction of waste oil management practice into the European legislation, with the publication the Council Directive on the disposal of waste oils (COUNCIL DIRECTIVE 75/439/EEC), even though document at that time related only to the lubricant oils waste. Since then this Directive has been amended on three occasions by the Directives on Amending

the Directive on the disposal of waste oils 1987/101/EEC, 1991/692/EEC and 2000/76/EC, and revoked by the Directive of the European Parliament and of the Council on waste and repealing certain Directives (DIRECTIVE 2008/98/EC). Current EC Directive on waste oil management covers all relevant provisions on waste oil management all of which have since been transposed into the legislation of the EU member states.

In terms of the environmental awareness, Croatia has never lagged far behind the advanced European counterparts. Thus, in 1982 it devoted great attention to waste oils in its Act on the disposal of waste materials (OG 42/82). Article 3 of the said Act classifies waste materials into four categories, namely: production and traffic waste, waste mineral and other oils, communal waste and unused energy. The same Act established the tributaries of the proscribed manner of managing the listed groups of waste, as well as the obligation to register economic subjects for the business activity of collecting waste oils.

Although the 1995 Waste Act (OG 34/95), which revokes the Act on the disposal of waste materials, does not mention waste oils expressly, opposed to packaging waste, it still is present in the implementation part of the regulation, which is the Ordinance on the kinds of waste (OG 27/96), placing great importance on waste oils.

This Ordinance, among other, determines the types of waste depending on their properties and place of generation, means of treatment individual waste types, manner of delivering information on waste management, testing physical and chemical properties of hazardous waste, sampling waste, etc., as well as proscribes in detail the manner of managing waste oils exclusively, while it lists general provisions for other waste types and categories. Furthermore, this document defines the term of waste oils, prescribes the classification according to the degree of pollution, defines the obligations of fresh oil producers and distributors, as well as waste oil producers, especially concerning collection (separate collection, specially marked tanks…), managing the register on generated quantities of waste oils, etc.

The 2004 Waste Act (OG 178/04) proscribed the obligation of drafting an ordinance which was to establish the treatment of all special waste categories.

The first in the series of respective ordinances was introduces in 2005 entitled Ordinance on packaging and packaging waste (OG 97/05, 115/05, 81/08, 31/09, 156/09, 38/10, 10/11, 81/11, 126/11, 38/13, 86/13), followed by Ordinance on waste tyre management (OG 40/06, 31/09, 156/09, 111/11, 86/13), Ordinance on waste oil management (OG 124/06, 121/08, 31/09, 156/09, 91/11, 45/12, 86/13), Ordinance on waste batteries and accumulators management (OG 133/06, 31/09, 156/09, 45/12, 86/13) and Ordinance on the management of end-of-life vehicles (OG 136/06, 31/09, 156/09, 53/12, 91/13) in 2006. 2007 saw Ordinance on the method and procedures for managing waste containing asbestos (OG 42/07), Ordinance on medical waste management (OG 72/07), Ordinance on the management of electrical waste and electronic appliances and equipment (OG 74/07, 133/08, 31/09, 156/09, 143/12, 86/13). Ordinances for other special categories of waste were adopted in 2008, namely: Ordinance on the construction waste management (OG 38/08), Ordinance on the management of wastewater treatment sludge when used in agriculture (OG 38/08), Ordinance on the management of waste from the titanium dioxide industry (OG 70/08) and the Ordinance on the management of polychlorinated biphenyls and polychlorinated terphenyls (OG 105/08).

Most of these ordinances have in the meantime seen a number of amendments, mostly relating to their harmonization with relevant EU legislations.

The objective of the Ordinance on waste oil management (OG 124/06, 121/08, 31/09, 156/09, 91/11, 45/12, 86/13) was to establish the system of waste oil collection for the purpose of disposal and/or recycling, environment protection in

general, as well as human health preservation.

This Ordinance proscribes the manner of managing waste oils, determines the tributaries of fees, types and amounts of those fees, payment manner and deadlines, amounts of fees payable to entities authorised for collection of waste oils, and many other issues relating to waste oil management.

Further, it also regulates the managing of register on quantities of products placed on the Croatian market (produced or imported), becoming a special waste category upon expiration. The provisions of this Ordinance do not refer to the managing of waste oil comprising biphenyls (PCB) and polychlorinated terphenyls (PCT) over 30 mg kg^{-1}, emulsions and oiled liquid waste having in its composition, apart from emulsion oils and other waste oils, other pollutants and water, as these issues are regulated by the Ordinance on the management of polychlorinated biphenyls and polychlorinated terphenyls (OG 105/08).

Since its first introduction, the Ordinance on waste oil management has seen numerous amendments, and it is to be replaced by a new Ordinance upon the adoption of a coming new Act on Sustainable Waste Management (OG 94/13).

Data on quantities of collected and disposed waste oils 1996-2004

The obligation of the Croatian waste producers on the manner of managing a register on types and quantities of generated waste in the form of Waste cadastre was regulated as far back as 1995 in the Waste Act (OG 34/95) and Ordinance on the kinds of waste (OG 27/96), while the system itself was implemented in 1997. Based on the 1994 Environmental Protection Act (OG 82/94), Regulations on Emission Inventory (OG 36/96) was adopted with the purpose of establishing a wholesome base of data on the environmental pollution via air, water and sea emissions from respective sources, as well as types and quantities of hazardous waste. Emission Inventory collected data on the environmental pollution from 1997 to 2008, when it was replaced by the environmental pollution register (OG 35/08) as a data base on the sources, types, quantities, manner and location of emissions, transfer and disposal of pollutants and waste into the environment.

Even though completed forms with data such as sources, types, quantities, manner and location of emissions, transfer and disposal of pollutants and waste into the environment were delivered to the county offices authorised for environmental protection, the available literature holds no systematized data on collected waste quantities 1996-2004. Until 2005 keeping the Waste cadastre was under the scope of (then) Ministry of Environment Protection, Urban Planning and Building. Since 2005 it is responsibility of the Croatian Environment Agency (OG 75/02), as regulated by the 2004 Waste Act (OG 82/94). This in turn means that the Croatian Environment Agency started receiving waste data for 2004 only in 2005. Since then Agency introduced the practice of reporting on Waste cadastre (WASTE CADASTRE, 2006; 2007; 2008.), and those reports, among others, presented the total number of the quantity of collected waste oil.

The first waste reports (WASTE CADASTRE, 2006; 2007; 2008.) usually presented total data for the whole Croatia in the first part, continuing with reported quantities of generated, collected and recycled production waste by categories with summaries of managing generated, collected and recycled waste in respective counties. The data delivered to the Waste cadastre were often incomplete and inaccurate, as reflected in wrong key numbers of reported waste, lack of information on waste flow, inadequate measuring units of the quantities of reported waste, etc. This is why the results of processing county and county level in those first reports should be regarded cautiously (Table 1).

The first detailed report with gathered data on waste lubricant oil for Croatia was in the 2005 Report (CROATIAN ENVIRONMENT AGENCY, 2007), listing that hazardous waste 13 00 00 (oil wastes and wastes of liquid fuels – except edible oils,

and those in chapters 05, 12 and 19), reported in the Waste cadastre in the amount of 9 461 tonnes represents as much as 26.6% of the total reported quantity of generated hazardous waste. Within the 13 00 00 group, the report listed the following: 2 853 t of sludge from the oil/water separator process by the solidification procedure, 2 457 t of non-chloride lubricant oils from motors and gears based on mineral oils mostly remaining at the place of collection, with only a minor part being sent for incineration, 1 094 t of other oiled waste temporarily stored at the place of collection, etc.

Table 1. Overview of quantities of generated waste oils as reported to the waste cadastre 2003-2006.

Waste oil, t	**Year**			
	2003	2004	2005	2006
Generated	13 303	11 258	9 461	10 083
Collected	NA	NA	10 409	15 292
Recycled	NA	NA	8 997	13 563

NA – not available

In the following year the share of waste oils in the total quantity of generated production waste was somewhat lower (CROATIAN ENVIRONMENT AGENCY, 2006; 2007; 2008) 25.3%. Of the totally reported 10 083 tonnes of 13 00 00 waste group, 2 530 t of non-chloride lubricant oils form motors and gears based on mineral oils mostly submitted to processing was reported, 2 003 t of sludge from the oil/water separator submitted for processing, 1 102 t of other oiled waste, etc.

Data on quantities of collected and disposed waste oils 2007-2012

Even though waste oil management, fee tributaries and fees themselves, etc. were proscribed in 2006 by the Ordinance on waste oil management (OG 124/06, 121/08, 31/09, 156/09, 91/11, 45/12, 86/13), the data on implementation and efficiency of its provisions could be analyzed only after the adoption of the 2007 Environmental Protection Act, under which the Croatian Environment Agency was obliged to draft annual Reports on the state of the environment in the Republic of Croatia.

The Ordinance on waste oil management set up the goal of establishing the system of collecting waste oils for the purpose of disposal and/or recycling, environment protection and people's health. Legislation also defined role of authorised collectors and recyclers, who were to engage in afore mentioned activities as legal entities and as such are obliged to obtain licences for the activities of collection, disposal and/or recycling waste oils. Pursuant to that, 50 Croatian business are in possession of licences for waste lubricant oil management (groups 12 01 and 13), and 88 businesses for waste edible oil management under the key number 20 01 25 (November 2013).

Until 2008 data on waste oil management were gathered based on the Regulations on Emission Inventory (EI) until the adoption of the Ordinance on the Environmental Pollution Register (OG 35/08), when Regulations on Emission Inventory stopped being legally valid.

The Ordinance on the environmental pollution register proscribes the obligatory contents and manner of keeping the environment pollution registry, the tributaries of delivering data to the registry, manner, methodology and deadlines for gathering and delivering data on emissions, transmissions and disposal of pollutants in the environment, as well as waste, data on polluting entities, company, plant, polluting entity's organizational unit, deadlines and ways of providing public information, information review and quality assurance, timeframe for archiving data and conducting expert activities regarding registry management.

This Ordinance fully ensures the implementation of the Regulation 166/2006 EZ on establishing the European Pollutant Release and Transfer Register, E-PRTR (REGULATION EC166/2006), and upon joining the EU, the Croatian Environment Agency is obliged to deliver data to E-PRTR. The first Waste Oils Report (ANNUAL CEA, 2008) for 2007 was drafted by the Croatian

Environment Agency pursuant to 2007 Environmental Protection Act and Ordinance on waste oil management. According to the mentioned report, during 2007, 31 478 t of fresh lubricant oil was produced and imported, i.e. placed on the market. In the same year 6 115 t of waste lubricant oil was collected, comprising 39% of the total of waste lubricant oil or only 19.4% of the fresh lubricant oil placed on the market.

In the first Waste Oils Report (ANNUAL CEA, 2008) further discusses the quantity of 1 132 t collected waste edible oil, even though data on the quantity of fresh edible oil placed on the Croatian market are not known, because the producers/importers of waste edible oil in Croatia are not tributaries of fees for waste oil management and are not under the obligation of delivering information on the quantities of fresh edible oil placed on the market to the Croatian Environmental Protection and Energy Efficiency Fund. Of the 1 132 t of collected waste edible oil, 691 t or 61% was delivered for processing. Amount of collected waste edible oil is much lower than the estimated quantity of produced waste edible oil from the Waste Management Plan of the Republic of Croatia (WASTE MANAGEMENT PLAN OF THE REPUBLIC OF CROATIA, 2007-2015; OG 85/07,126/10, 31/11) for 2007-2015, estimated at 50 000 t year^{-1}.

These first reports on waste oil management, based on the data submitted by the authorised collectors and recyclers to the Croatian Environmental Protection and Energy Efficiency Fund and Croatian Environment Agency saw certain data discrepancies. This was a direct result from poor defining of key numbers of some of the waste from 13 00 00 group, levelling litre and kilogram units, etc. For instance, in the 2007 report five of total twenty authorised collectors reported the same data to the Croatian Environmental Protection and Energy Efficiency Fund and Croatian Environment Agency, thus the said discrepancies come as no surprise at all.

Regulation on categories, types and classification of waste with a waste catalogue and list of hazardous waste (OG 50/05, 39/09), includes and summarizes waste oils covered by the Ordinance on waste oil management in chapters 12, 13 and 20. Same classification is used for the regular annual reports on waste oils drafted by the Croatian Environment Agency, alongside with waste oils from chapter 13 00 00, namely oil wastes and wastes of liquid fuels – except edible oils, and those in chapters 05, 12 and 19. Report also contains data on managing other waste like:

- 12 01 06* - mineral-based machining oils containing halogens (except emulsions and solutions);
- 12 01 07* - mineral-based machining oils free of halogens (except emulsions and solutions);
- 12 01 10* - synthetic machining oils;
- 12 01 12* - spent waxes and fats and
- 12 01 19* - readily biodegradable machining oil.

During the last six years of implementing the Ordinance on waste oil management, no significant change in waste oil management has been reported. The quantities of fresh lubricant oil placed on the market of Croatia went from 21 219 t (2012) to 35 576 (2008), and the result are the development of produced, imported and exported quantities, as presented in Table 2.

From the data (ANNUAL CEA, 2008 – 2012; DATA ON WASTE OIL, 2012) shown in Table 2, it is evident that in the period 2007-2012 the annual amount of collected waste was almost equal, and that the quantities of waste lubricant oil went from 6 115 t (2007) to 7 068 t (2008), while a drop in the quantity of collected waste lubricant oil was reported in 2009. In the same period almost the entire collected quantity was recycled, and Table 2 demonstrated that in the first years of implementing the Ordinance on waste oil management and monitoring waste oil management, the shown quantities of recycled waste oil were sometimes bigger that the shown quantities of collected lubricant oil (2007-2009), resulting from the manner of delivering incomplete data by the licensed companies. According to data shown in Table 2, in the 2007-2012 period, between 20 488 t (2010) and 35 576 t (2008) of fresh lubricant oil was placed on the Croatian market, while between 5 835 t

(2012) and 7 068 t (2008) of fresh lubricant oil was collected, i.e. between 39% (2007) and 65% (2010) of estimated produced quantities of waste lubricant oil, usually comprising around one half of the annual quantity placed on the market (WASTE OILS, 2013), as shown in Fig. 1.

As the Ordinance on waste oil management implies both lubricant waste oils and edible waste oils under the term waste oils, in the annual reports (ANNUAL CEA, 2008 – 2012; DATA ON WASTE OIL, 2012) due attention was also paid to waste edible oils.

Waste edible/cooking oil (20 01 25 - edible oil and fat) is, pursuant to this Ordinance, any oil produced within catering and tourist-related activities, industry, craft, health-related activities, public management and similar businesses preparing over 20 meals per day, meaning that most provisions from the Ordinance apply to these waste oils as well.

According to the data on collected waste edible oil in the 2007-2012 period in Croatia, it is evident that the collected qualities are very small relative to estimated annual amounts of fresh edible oil placed on the market of 50 000 t or supposed quantities of waste oil, estimated at 50% of the total fresh oil quality (Table 3).

Table 2. Overview of the development of lubricant oil in Croatia 2007-2012.

Lubricant oil, t	**Year**					
	2007	2008	2009	2010	2011	2012
Produced fresh oil	12 501	12 413	10 417	7 031	9 205	6 723
Imported fresh oil	23 794	28 518	24 212	22 939	25 095	22 253
Exported fresh oil	4 816	5 355	10 567	9 482	9 633	7 757
Placed on the market fresh oil	31 478	35 576	24 062	20 488	24 667	21 219
Collected waste oil	6 115	7 068	6 785	6 640	6 391	5 835
Recycled waste oil	6 364	7 131	6 843	6 535	5 906	5 125

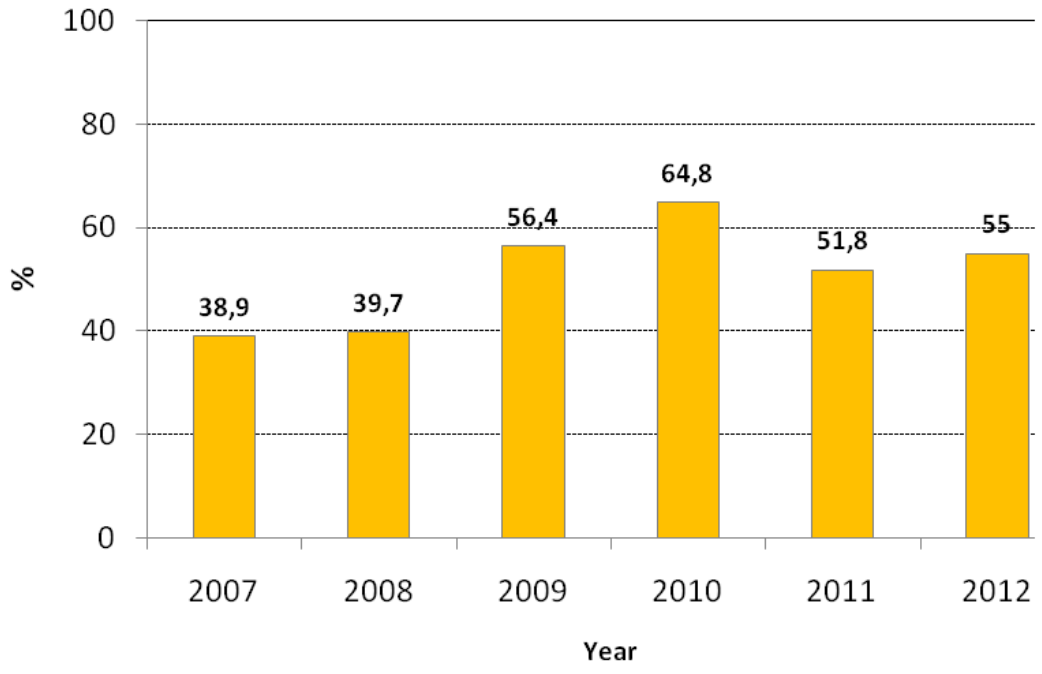

Fig. 1. The share of collected waste lubricant oil in the estimated produced quantity of waste lubricant oil in the 2007-2012 period.

Table 3. Overview of the development of waste edible oil in Croatia 2007-2012.

Edible oil, t	Year					
	2007	2008	2009	2010	2011	2012
Placed on the market fresh oil	50 000*	50 000*	50 000*	50 000*	50 000*	50 000*
Collected	1 132	1 606	2 145	1 260	1 196	911
Recycled	691	1 033	1 507	683	744	678

*Estimation (WASTE MANAGEMENT PLAN OF THE REPUBLIC OF CROATIA, 2007-2015; OG 85/07,126/10, 31/11)

According to data presented in Table 3, and based on the comparison of data on collected quantities of waste edible oil and estimated annual quantities of fresh edible oil placed on the market of 50 000 t, it ensues that in the 2007-2012 period, very little, i.e. between 3.6% (2012) and 8.6% (2009) was collected relative to the estimated quantity of the total collected waste edible oil (Fig. 2). Having in mind that, according to the data of the Croatian Bureau of Statistics for the 2005-2011 period, the annual per capita consumption of edible oil (CROATIAN MAIN CHARACTERISTICS HOUSEHOLD CONSUMPTION AND HOUSEHOLD, 2007 – 2011) was between 13.1 and 14.6 litres, implying that the annual quantity of fresh edible oil placed on the market may be higher than the estimated 50 000 t, in that case the real results on annually collected waste edible oil are even poorer than reported.

To be precise, the European and global statistics report that 40-50% of edible oil used for food preparation is consumed by restaurants and in the food industry, whereas the rest is consumed by household, of which 50% in food and the rest presents waste edible oil (CVENGROŠ *et al.*, 2004; KLEINOVA *et al.*, 2009; ADEME, 2010). According to that, in the EU countries, the total quantity of produced waste edible oil is 5kg per capita annually. In the same period between 678t (2012) and 1507t (2009) was recycled, i.e. between 54.2% (2010) and 74.4% (2012) of the total quantity of collected waste edible oil, as presented in Fig, 3.

Comparing the results of quantities of collected and recycled waste edible oil in 2008 with data available from the literature (CVENGROŠ *et al.*, 2004; KLEINOVA *et al.*, 2009; ADEME, 2010) for the same year in some EU member states, it is evident that the results of collection and recycling of waste edible oil in Croatia are significantly below the results achieved during the same year in the observed EU member states, as presented in Table 4.

Table 4. Waste edible oil management in some EU countries and Croatia in 2008.

Country	Edible oil, t			Recycled, %
	Fresh oil placed on the market	Generated waste oil	Collected waste oil	
Belgium (BE)	110 742	46 710	44 711	94
Denmark (DK)	61 182	28 000	20 000	100
Croatia (HR)	**50 000***	**25 000***	**1 606**	**64**
Italy (IT)	529 870	227 371	212 497	71
Germany (DE)	1 079 576	493 000	493 000	100
Portugal (PT)	93 576	41 169	31 695	89
Spain (ES)	485 200	190 000	180 000	100

*Estimation (WASTE MANAGEMENT PLAN OF THE REPUBLIC OF CROATIA, 2007-2015; OG 85/07,126/10, 31/11)

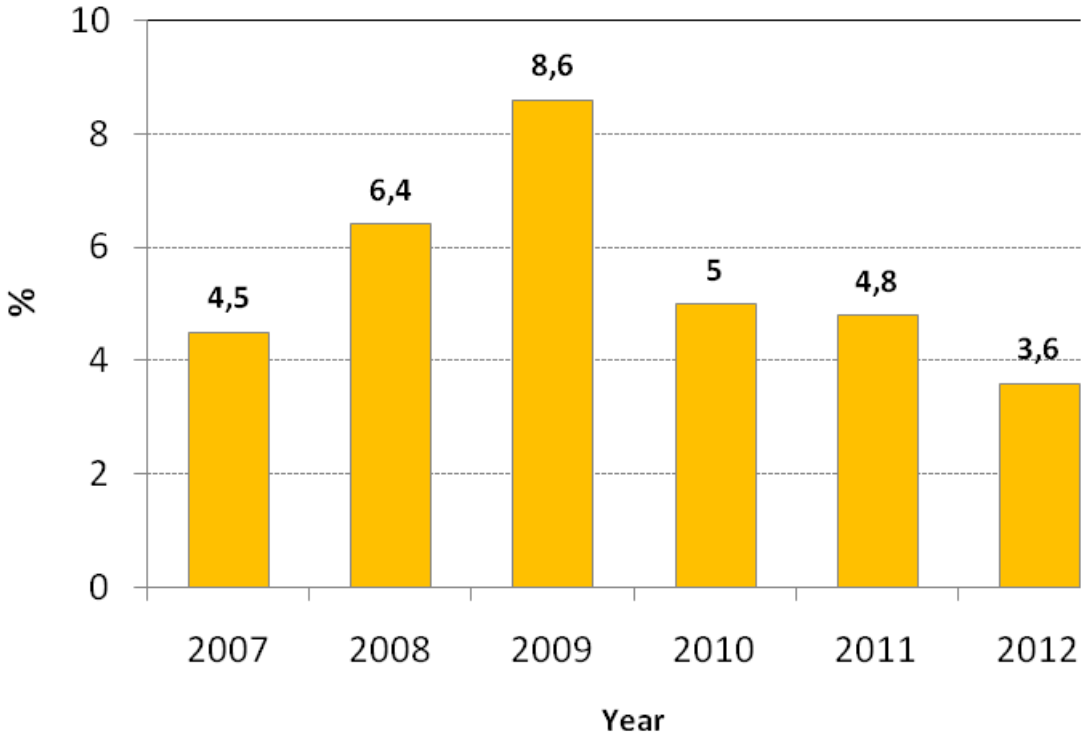

Fig. 2. The share of collected waste edible oil in the estimated produced quantity of waste edible oil in the 2007-2012 period.

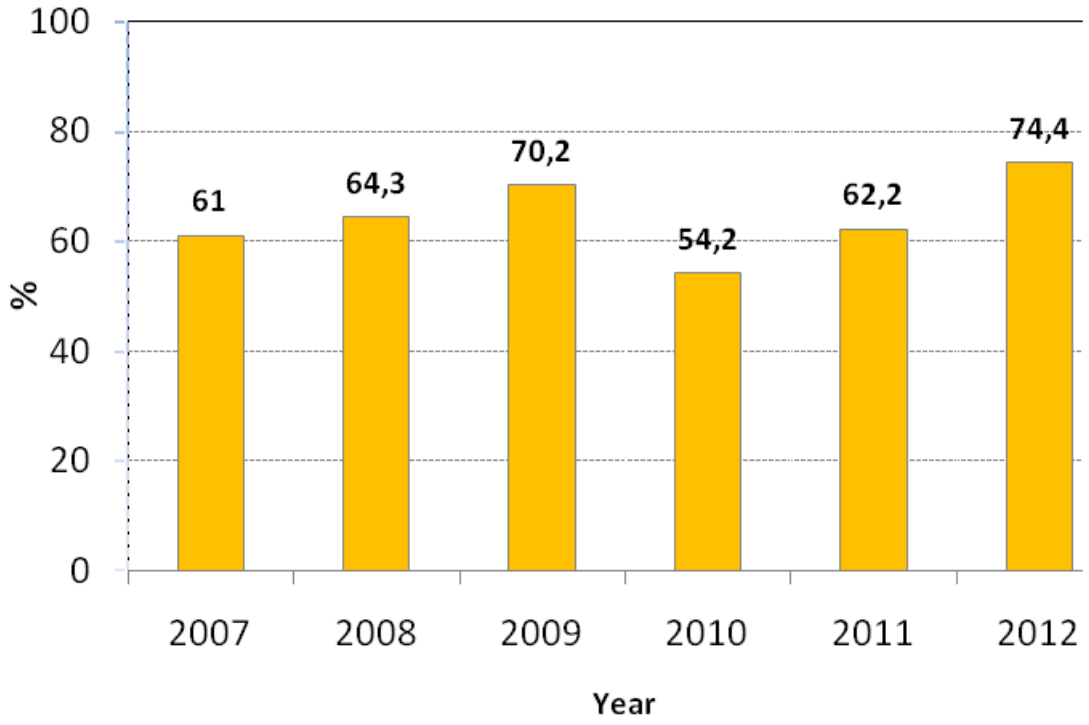

Fig. 3. The share of recycled waste edible oil in the estimated produced quantity of waste edible oil in the 2007-2012 period.

Conclusion

This paper has described the course of introducing and implementing the regulations ensuring simultaneous learning, establishing and implementing waste oil management system. At the same time, it shows Croatia's commitment for a general consideration of this segment of environment protection and human health, resulting in existing legislature and long experience in reporting on waste oil management results.

According to the results demonstrated for 2007-2012 between 5 835 t (2012) and 7 068 t (2008) of waste lubricant oil was collected in Croatia, i.e. between 39% (2007) and 65% (2010) estimated generated quantities of waste lubricant oils.

According to the data on quantities of collected waste edible oils from 2007 to 2012 in Croatia, one may conclude that those quantities are very small relative to the estimated annual amounts of fresh edible oil placed on the market (50 000 t), i.e. supposed quantities of waste edible oil, from 3.6 % (2012) to 8.6% (2009).

From 2007 to 2012 almost entire quantity of collected waste lubricant oil was recycled, and the share of recycled waste edible oil was between 54.2% (2010) and 74.4% (2012) of the total quantity of collected waste edible oil. Thus it is important to note that the quantities of collected waste edible oil in Croatia are very small and it is essential to make steps towards improving significantly the system of waste oil management relating to edible oil.

In general, the share of recycled waste oil is very difficult to comment in relation to the objective determined by the Waste Management Strategy of the Republic of Croatia (OG 130/05), which was to be met as far back as 2010. To be more precise, the 90% waste oil recycled goal determined by the Strategy has not been clearly defined, i.e. it is not clear whether it refers only to recycling lubricant oils or edible as well, and whether 90% of collected or generated waste oil is to be recycled. On the EU level, recycled quantities of waste oil are expressed in the relation to collected quantities.

Regardless of the inconsistencies in terms of the Strategy's objectives, the results of waste oil management, both lubricant and edible, are not significant, which leads to the conclusion that, based on the comparison of demonstrated results relating to the collection and the recycling of waste lubricant oil and waste edible oil in Croatia and some EU member states, Croatia needs to continue with the ongoing education of all participants, especially small consumers like households with the purpose of improving the system of waste oil management.

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Software:

STATSOFT INC. 2004. STATISTICA (Data analysis software system), Vers. 7. Computer software. [http://www.statsoft.com].

GARMIN LTD. 2007. MapSource, Vers. 6.12. Computer software. [http://www.garmin.com]

Website:

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