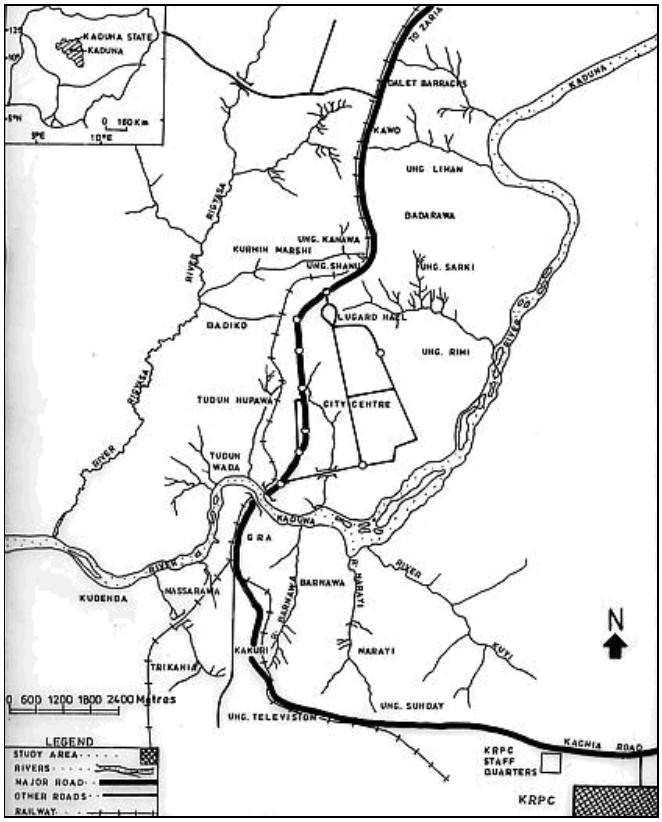
Applied Ecology and Environmental Research

International Scientific Journal



VOLUME 6 * NUMBER 3 * 2008

http://www.ecology.uni-corvinus.hu
ISSN 1589 1623

AN APPROACH FOR IMPACT ASSESSMENT OF TRANSGENIC PLANTS ON SOIL ECOSYSTEM

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(Received 28th November 2006 ; accepted 12rd March 2007)

Abstract. Transgenic crops are new products of agriculture biotechnology. The environmental risks and benefits of transgenic crops are topic of hot debate. Current agriculture management practices and ecosystems have their own impacts on the environment and further any additional negative effect of transgenic crops may mitigate their positive impacts as well as increase the background value of negative impacts due to new agriculture practices. Most of the risk assessment studies on transgenic plants have done observations on changes in their respective aboveground environment and its biota. Very few reports are available on the impacts of transgenic plants or their products (that they release in soil) on soil biota (both invertebrates and. microorganisms) and soil processes mediated by them. However, observations of these studies were not delivering anything conclusively and creating state of confusion also regarding impact of transgenic plants on soil ecosystem. As some of the studies suggested that If production and release of the transgene products from transgenic plants through different routes in soil exceed to its consumption/ biodegradation, may lead to their accumulation beyond threshold levels, which may have acute as well as chronic effect on soil ecosystem. Impacts of transgenic plants are also dependent upon spatial and temporal environmental variables. Whereas some of the studies observation suggests that transgenic plants don't have any negative impact on soil ecosystem. Keeping this status in background we prepared this manuscript. Our manuscript is divided in two parts, first part comprises review of the available literature on impacts of commercialized transgenic plants on soil ecosystem and its diversity, and in second part keeping above information as background, a framework is proposed for future comparative impact assessment of transgenic plants and its non transgenic isoline on soil ecosystem. In this approach each transgenic crop along with its non-transgenic isoline should be dealt separately according to its construct. The proposed approach is precautionary at each step, if there is any doubt at any stage they should be clarified by repetition of experiments. This approach will be helpful in filling of information gaps, which still exists in impact assessment studies of transgenic plants on soil ecosystem. This approach suggested monitoring should be carried out prior as well as post release of transgenic plants. Impact assessment of transgenic plants with respect to soil ecosystem should be made mandatory in current regulatory framework of transgenic crops throughout the world, to assure the use of transgenic technology without affecting the diversity and functioning of soil ecosystem.
Keywords: *Transgenic, soil, impact assessment, ecosystem*

Introduction

One key challenge for the twenty-first century is to provide food security to growing population of the world. This challenge has led agriculture sector towards gene revolution after green revolution with the help of advanced biotechnology. It has been proclaimed as third technological revolution following the industrial and computer revolution [1]. The gene revolution of agriculture involve understanding and modifying the organization of traits within the chromosomes of the species and conversion of traits of an organism by transferring individual genes from one species to another i.e. creation of transgenic.

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First developed transgenic plants, in early 1980s were tobacco [30] and petunia [33] having trait for resistance to the antibiotic kanamycin. Since then, numbers of transgenic crops were developed to achieve novel and desired traits. The purpose of creation of transgenic plants are profound such as pest resistance, herbicide tolerance, long shelf life, phytoremediation, vaccine production, nutrient supplement and tolerance to biotic and abiotic stresses.

Number of countries cultivating transgenic crops commercially have increased from one in 1992 to twenty one in 2005. The estimated global area under transgenic crop cultivation till 2005 was approximately equivalent to 222 million acres [41]. The major traits harbouring transgenic crops were herbicide resistance (HT) with 72% of the total transgenic area followed by insect resistance (IR) with 19.5% and both HT and IR stacked in one crop occupied only 8.5%. Whilst, virus resistance and other traits engaged very little area <1%. The principal commercialised transgenic crops are soybean (48.4m ha), corn (19.3mha), cotton (9.0 mha), canola (4.3 mha) alfalfa and potato (<0.1mha), (James, 2005). Other non-commercialized but ready for trial transgenic crops are papaya, squash, rice, bringal, aubergine, sugarbeet and tomato. More crops under development in labs for being transgenic are apple, mango, banana, pineapple, barley, sweetpotato and coconut.

Transgenic plants contain foreign genes responsible for release of compounds, which help them in growth and establishment outside their natural habitat, with enhanced survival, persistence and competitive capabilities. Now a days several ecological concerns are arising with respect to the cultivation of the transgenic crops such as - invasiveness, gene flow to indigenous organisms, development of resistance in target pests, and direct or indirect effects on nontarget organisms and ecosystems [72, 73]. Among direct or indirect effects of transgenic plants, concerns about their impacts on soil ecosystem have been raised due to the chemical and biological properties of soil [54]. Soil materials have large sorptive capacities for biological molecules, including DNA and insecticidal bacterial proteins. There is increasing scientific data to show that soil can, in some cases, protect these molecules from biological degradation, enabling their toxic properties and genetic information to be retained in soil for unexpectedly long periods.

The impact of transgenic plants on non-target soil microorganisms will depend on the nature of the recombinant protein (i.e. its spectrum of activity) and the extent of exposure. Some transgenic plants (insect resistant) change its rhizosphere environment through root exudates, which consequently promote or retard the growth of microorganisms in the rhizosphere [54, 68]. Transgenic Bt cotton and Bt corn plants release Bt endotoxin into the soil from their different parts (roots, leaves etc), which persists in soil and retained its immunological and biological activity [64, 66]. A major problem in evaluating the impacts of transgenic crops on soil microbial diversity is the lack of baseline information on- diverse agroecosystems to compare with agroecosystems in which transgenic crops have been introduced [10, 17], and lack of universally approved approach for carrying out impact assessment of the transgenic plants on soil ecosystem. Consequently, it is important to review literature for the systematic understanding of risks of transgenic plants and their products observed till now on soil microbial diversity as well as to propose a framework based on approach which should be followed worldwide for impact assessment of transgenic crops on soil ecosystem at various regulatory stages prior to their release as well as after their commercialization.

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Interaction between transgenic plants and soil microorganisms

Soils are among the most species rich habitats on the planet Life in soils includes vertebrates, macrofauna, micro- and mesofauna as well as algae, lichens, protozoa, fungi, bacteria and viruses (*Figure 1.*). Soil diversity is often several orders of magnitude greater than that of which occur aboveground [40]. Microbial diversity of soil play important role in maintaining its resiliency [55].

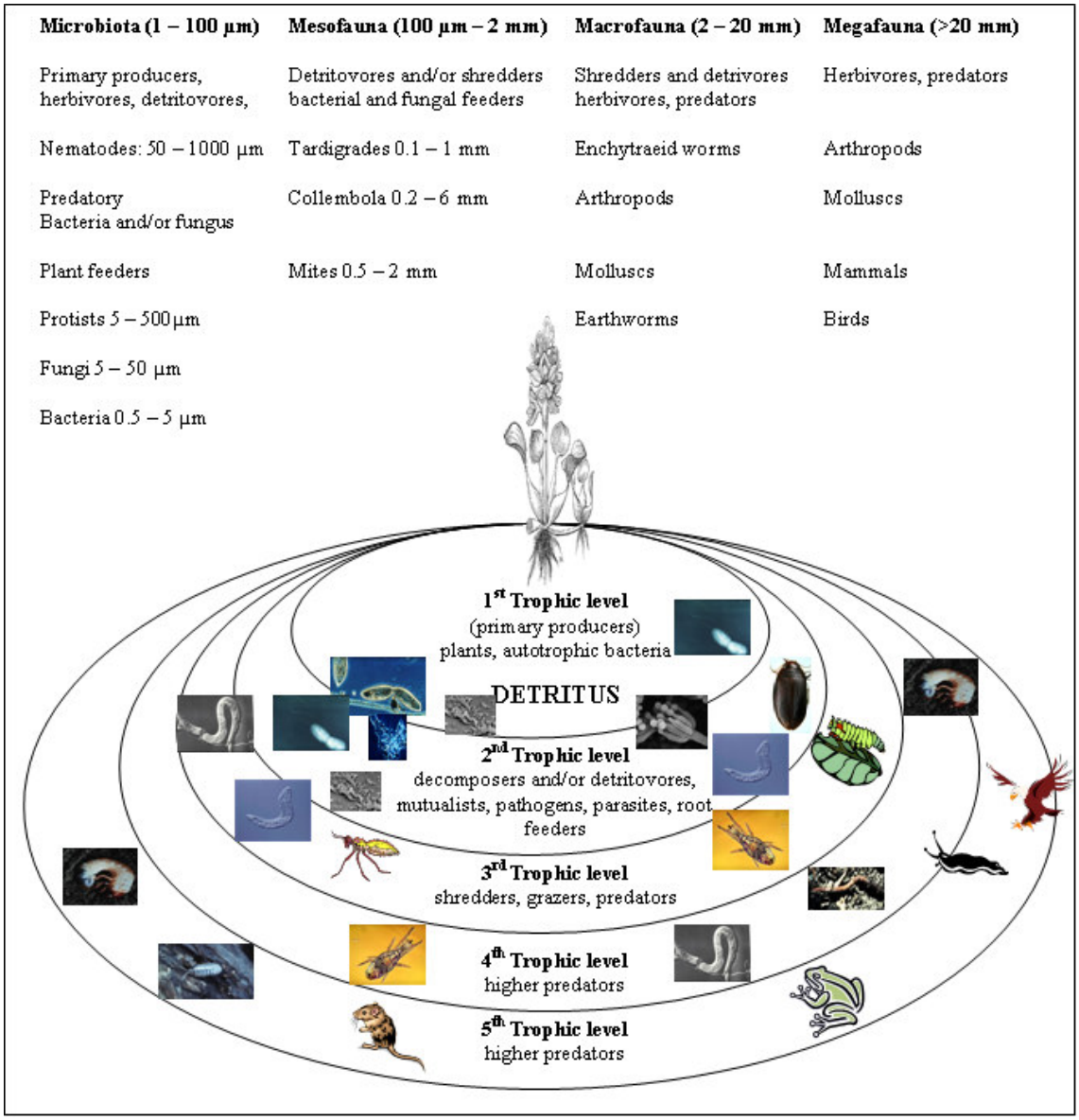

Figure 1. *Soil food web showing an outline classification of the soil biota based on type, body size and trophic level. These components have variety of interaction ranging from the competitive or predatorial to the cooperative and symbiotic. Size class is based on the width of the organism according to Swift [78].*

Biodiversity of soil provides it an insurance against environmental perturbations, because different species or communities respond differently to these fluctuations, leading to more stability of ecosystem properties [47].

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Addressing the impact of transgenic plants on a soil ecosystem is an important issue but distinctions must be made between an impact on soil microbial diversity (the range of organisms the soil contains) and one on soil function (what the soil does?). Reduction in soil microbial diversity might not necessarily cause a decrease in soil function. Studies investigating the links between diversity and function have reported both- a strong role for diversity in soil function [80], and also that function can be maintained with just a relatively small number of species present [47]. The general consensus among researchers is that there is 'no predictable relationship between diversity and function' and that species richness is not a dominant factor in the overall functioning of soils [9].

Plants are the main drivers of soil ecosystem and soil provides spatially and temporally heterogeneous environment to plants and soil microbes. Plants influence soil through rhizodeposition (root exudates, sloughing of root cells and root turnover), plant litter, water, gas and nutrient exchange. Interaction between soil microorganisms and plant roots satisfy important nutrient requirements for both the plants and the associated microorganisms [12]. Interactions between transgenic plants and soil biota occur naturally as well as through anthropogenic activities.

Natural interactions

Naturally soil microorganisms interact with plant roots on the rhizoplane and within the rhizosphere[43],. In rhizosphere, plant roots have direct influence on the composition and density of soil microorganisms through rhizospheric effect. Normally microbial population is large and more active in rhizospheric zone as compared to bulk soil. Not only physical and chemical conditions are different in the rhizosphere, but plant roots also provide the majority of substrate for energy and biosynthesis of microorganisms in soil. Plant roots release carbohydrates, aminoacids, organic acids, lipids, hormones, vitamins, enzymes, mucilages, mucigel, and lysates into the rhizosphere as root exudates [43]. Plants can therefore through its rhizosphere have a profound effect on soil-borne microbial communities and processes; vice versa soil microorganisms affect plant growth and performance through their activities.

Naturally, transgenic plants according to introduced transgenic trait releases different transgene products (Bt toxin and T4 lysozyme) at different growth stages into the soil ecosystem actively via different routes such as- from the roots as root exudates, via leachates following plant injuries, from sloughing off root / root cap cells and through the decomposition of senescent leaves and left over transgenic plant biomass in field after final harvest [22]. It has been reported that Bt endotoxin released from root exudates (as well as from pollen and crop residues) binds rapidly to montmorillonite and kaolinite clay minerals, humic acids, and organomineral complexes [14, 15, 69, 79]. The soil bound toxin retains its activity and has been observed to persist in soil for up to 140 days [65] to 234 days [68]. Palm[65] observed a general pattern of rapid degradation of the toxin during the first 14 days of incubation followed by a slower rate of degradation over the total 140-days study period.

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Anthropogenic intervention

Interaction between transgenic plants and soil biota is also influence due to anthropogenic interventions. These anthropogenic interventions are man managed agriculture practices and includes- tillage activities, number of pesticide or herbicide and irrigation applications etc. Transgenic crops residue incorporated in soil through tillage practice, which during its biodegradation interact with soil biodiversity and influence it either positively or negatively. In zero-tillage practice, crop residues are left concentrated on the soil surface, which limits the soil microorganisms to come in contact with the protein at the soil surface. Whereas in conventional tillage, the plant litter incorporated into the soil, diluting the concentration of the transgene products but increasing the number of organisms exposed [6]. Zwahlen [86] also observed variation in long-term persistence of the Bt toxin from transgenic corn residues in field trials with respect to tillage practices. The presence of transgene products in soil may influence the structure and function of the microbes and their community by selectively stimulating or arresting the growth of organisms that can use them.

Effects of transgenic plants on structure and functions of soil microorganisms

The introduction of transgenic crops and their novel products in soil ecosystem may alter structure of soil microorganisms directly or indirectly. Most of the risk assessment studies on transgenic plants have tried to address the impact of commercialized transgenic plants on the structural alteration of soil microbial community as compared to its non-transgenic isoline. The observed and reported impacts of transgenic plants on structural pattern of its soil microbial community are listed in (*Table 1*). Donegan [20] examined the effects of decomposing transgenic cotton litter on structure of soil microbial communities and observed transient and significant increase in culturable aerobic bacteria and fungi in two out of three transgenic lines, which was attributed to unexpected changes in plant root exudates. Similarly, aerobic bacterial, fungal population and fungal species diversity in soil samples of Bt potato (*Solanum tuberosum* L.) differ significantly from non-Bt potato plants [21]. Soil from fields of transgenic alfalfa (herbicide resistant) has shown significantly higher population levels of culturable, aerobic spore-forming and cellulose-utilizing bacteria compared with that of the parental non-transgenic lines [23]. Lottmann and Berg [50] in Germany analysed rhizobacterial population of transgenic potato crop (expressing T4 lysozyme against the bacterial pathogen *Erwinia carotovora*) and nontransgenic potato and found that many bacterial and fungal species are sensitive to T4 lysozyme in vitro, but significantly high colony counts of T4-lysozyme tolerant Pseudomonas putida (potential biocontrol bacterial strains) were recovered from the transgenic plants than from control plants [50]. Donegan et al. [22] also found differences in population size of nematode and Collembola and earthworm in the soil surrounding the transgenic tobacco plant (expressing Proteinease Inhibitor I) litterbags. Wherease Saxena and Stotzky [69] observed no apparent effects of Bt toxin from corn added to soil on earthworms, nematodes, protozoa, bacterial and fungal population. However, they suggested that more detail research should be conducted to determine the effects of the toxin on soil biodiversity.

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Table 1. *Effect of transgenic plant on structure and functions of soil microorganisms and their communities*

Plant	Transgenic trait	Effect on soil biota	Reference
Cotton	Insect resistance	Significant stimulation in growth of culturable bacteria and fungi with change in substrate utilization	[20]
Rape	Herbicide resistance	Community fatty acid, community level physiological profile altered, taxonomic diversity of root associated community altered, altered diversity of *Rhizobium leguminosarum* and variation in Pseudomonas population	[37, 74, 75]
Soybean	Herbicide resistance	Incidence of Fusarium (soilborne pathogen) on transgenic soybean roots was greater within 1 wk after the application of glyphosate as compared to non-transgenic isoline.	[45]
Tobacco	Insect resistance	Alteration in community size of nematodes and Collembola	[22]
Potato	Insect resistance	Altered CLPP pattern of microbial community in transgenic rhizosphere	[36]
Wheat	Pathogen resistance	Variation in cultivable rhizospheric community	[58, 59]
Alfalfa	Organic acid expression	Qualitative changes in the abundance of bacterial phylogenetic groups between rhizosphere soils of transgenic and untransformed alfalfa. rhizosphere of transgenic alfalfa had significantly greater microbial functional diversity compared with untransformed alfalfa.	[80]
Lotus	Opine production	Increased population of opines utilizing bacteria as compared to other bacterial species in the rhizosphere of transgenic lotus.	[61]
Potato	Expressing the phage T4 lysozyme gene	Transgenic potato plants root are showing high bactericidal activity against *Bacillus subtilis* adsorbed artificially on potato roots as compared to nontransgenic plants	[2]
Potato cv. Desiree and harbor	T4 lysozyme-producing plant lines DL4 and DL5	No difference in growth of bacterial communities was observed between the rhizosphere of transgenic potato and non-transgenic potato varieties.	[39]
Rice	Insect resistance	Differences in protease, neutral phosphatase and cellulase activities between soil amended with Bt-transgenic rice straw and non-transgenic rice straw were not persistent. However, differences in dehydrogenase activity, methanogenesis, hydrogen production and anaerobic respiration were persistent.	[85]
Maize	Insect resistance	No significant differences in earthworm, microarthopods, nematodes and protozoan population due to exudation of Bt toxin in rhizosphere of Bt corn as compared to non Bt corn.	[4, 69]
Tobacco	Expression of proteinase inhibitor I	Number of collembella colonies associated with transgenic tobacco litter are less as compared with non transgenic litter. Whereas nematode population is high in transgenic litter as compared to non-transgenic litter.	[22]

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Plant	Transgenic trait	Effect on soil biota	Reference
Rape var. Quest	Herbicide resistance	rhizosphere and root interior microbial populations associated with a transgenic canola have altered CLPP and fatty acid methyl ester (FAME) profiles compared to the profiles of a nontransgenic counterpart.	[25]
Oilseed rape	Herbicide resistance	From root interior and rhizosphere fewer Arthrobacter, Bacillus, Micrococcus and Variovorax isolates, and more Flavobacterium and Pseudomonas isolates were found in the root interior of Quest compared to Excel or Parkland. The bacterial root-endophytic community of the transgenic cultivar, Quest exhibited a lower diversity compared to Excel or Parkland.	[75]
Maize	Herbicide resistance	No effect of transgenic maize was observed on genetic diversity of bacterial communities in rhizospheric samples.	[71]

Variations in response of soil diversity with respect to varieties and environment conditions of transgenic plant have been observed in few studies. Heuer [39] reported that the rhizosphere community structure associated with DL 4 line of transgenic potato was different than the community structure associated with a DL5 transgenic line and the control (DES). But contradictory to Heuer, Christopher and Jeffrey [13] observed rhizospheric and bulk soil samples of Bt corn for PLFA and bacterial and fungal CLPP profiles and found 73% and 6.3 -3.8% differences respectively of rhizospheric and bulk soil microbial community. But they found that expression of Cry endotoxin and varietal differences of transgenic corn don't have a significant effect on microbial profiles, except in the high-clay soil where both factors significantly affected bacterial CLPP profiles (accounting for 6.6 and 6.1% of the variability). The persistence as well as intensity of the impacts of transgenic plants on the structure of microbial community is dependent on the environment conditions of cultivation site [25, 26, 36]. Terminal restriction fragment length polymorphisms (T-RFLP) patterns of microbial communities associated with the rhizosphere of transgenic plants varies with spatial and temporal effects and spatial by temporal interactions [51]. Reports on retention time of transgenic products in soil are very limited. Head [38] reported that Cry1Ac protein was undetectable from soil samples of six fields that are under cultivation of Bt cotton from last 3-6 yrs and have incorporated Bt cotton plant residues by postharvest tillage. Wherease Oger et al. [62] observed that changes induced by transgenic plants in soil microbial community structure are persistent for a long time.

Above studies observations has shown variation in response of soil microorganisms to various transgenic plants due to different reasons. Spatial and temporal factors of cultivation site play an important role in determining the impact of transgenic plants on soil ecosystem. Any changes in soil due to introduction of transgenic plants should be crosschecked by replication of the experiment over the long duration. Very few reports are available on the effect of transgenic plants on invertebrate or protozoan population of soil. Experimental studies are urgently needed on the structural alterations of invertebrate or protozoan population of soils due to transgenic plants. Further research for probing non targeted traits of new transgenic crops and the mechanisms by which these traits may affect soil biological structure and processes, including changes in

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composition of root exudates, is needed for better understanding of the potential impact of transgenic crops on soil ecosystem.

The major functions of soil microbes in soil ecosystem are to carry out energy flow and nutrient cycling. Energy flow always takes place from primary producers to primary, secondary and tertiary consumers. In soil ecosystem this flow will takes place from plants to microorganisms (decomposers and saprophytes). The energy flow in soil ecosystem may be affected if transgenic plants will negatively or positively alter structure and composition of soil microbial community. Energy flow in any ecosystem is mediated through food chains or on larger scale through food webs. Positive or negative impact of transgenic plants on a single link of the soil food web will affect the energy flow. Reports on the energy dynamics in soil ecosystem with respect to transgenic plants are lacking. So this information gap should be filled by future research on transgenic plants and soil ecosystem.

The two major pools of soil nutrients are- the mineral (the clay fraction) and the organic matter. Organic matter part of soil nutrients is composed of decomposed plant and animal residue. Nutrient turnover from residues of transgenic crops as compared to non-transgenic isolines may vary because of the following reasons (i) alterations in the composition, quantity, and physical form of residues from transgenic crops; (ii) inhibition or stimulation of soil microbial communities involved in nutrient transformations, possibly through chemical compounds contained in the transgenic residues; and (iii) changes in management practices (e.g., tillage) for transgenic crops, which affect biotic and abiotic factors involved in decomposition and nutrient turnover of crop residues.

Differences in the composition of crop residues due to the introduction of a transgenic trait have been primarily observed in transgenic Bt crops. Masoero [52] reported that two transgenic Bt corn hybrids had higher starch, lignin and lower protein and soluble nitrogen contents compared with non-transgenic corn. Saxena and Stotzky [70] also observed 33 to 97% higher lignin content in 10 Bt corn hybrids, compared with their respective non-Bt isolines. In contrast, Escher [28] found low carbon: nitrogen ratio (C / N), lignin content and higher content of soluble carbohydrates in the leaves of one Bt corn variety compared with the corresponding non-transgenic corn variety. The effects of crop residue composition and the importance of several indices of residue quality, including the C/N ratio, lignin content, lignin / N ratio, initial N content, polyphenol content, polyphenol /N ratio, and initial soluble C concentrations of the residue, on decomposition and N mineralization have been extensively examined under both temperate and tropical conditions [29, 32]. In general, as the lignin or polyphenolic content / N ratio of the plant material increases it will slow down the decomposition rate of the crop residue and reduce short-term N availability [29]. Therefore, the lower or higher lignin content observed in some Bt crops may affect rate of residue decomposition and N mineralization of organic N contained in the residues. However, any reduction in the rate of N mineralization due to transgenic residues could offset normal nitrogen cycle of soil ecosystem.

Inhibition or stimulation of growth of soil microbial communities through chemical compounds released from the transgenic residues may affect their normal functioning is evidenced by changes in enzymatic activity. Wu and Min [85] observed protease, dehydrogenase, neutral phosphatase and cellulase soil enzymes activities in soil amended with Bt-transgenic rice straw and non-transgenic rice straw and reported high dehydrogenase activity in the transgenic straw amended soil compared to the control,

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with the differences persisting over 80 days. Whereas the activity of dehydrogenase and alkaline phosphatase soil enzymes in transgenic alfalfa (herbicide tolerant) rhizosphere was low as compared to its nontransgenic isoline [23]. As phosphatase enzymes were involved in soil organic phosphorous mineralization and plant nutrition, and dehydrogenase enzyme play important role in biological oxidation of organic compounds and also reflect the total viable microbial population responsible for decomposition of organic matter [31]. Alterations in soil enzymes activity shows the indirect impact of transgenic plants on cycling of nutrient in soil.

Studies are very limited on the response of soil microbe's to transgenic plants under sustainable and conventional agricultural practices. Only few studies have reported impact of different tillage practice with respect to transgenic plants and their impact on soil ecosystem [6]. Sharon [77] observed the decomposition of the transgenic Bt cotton and glyphosate tolerance (roundup ready) cotton crop residue within agricultural systems under conventional-tillage (CT) and no-tillage (NT) management, and found that the mass loss with subsurface decomposition of transgenic cotton residue in the CT reached 55% but surface decomposition in the NT reached only 25%. Under CT more transgenic biomass will come in contact of soil microbial diversity, which may affect its structure and function. Wherease NT causes deposition of transgenic crop biomass on the soil surface prevents soil erosion. In addition, improved weed control with herbicide-tolerant crops has also stimulated a larger proportion of producers to adopt conservation tillage leaving more crop residues on the surface and potentially reducing soil erosion [3, 56]. Transgenic crops (herbicide tolerant) could support a reduction in tillage through direct drilling into a weedy field, which may be beneficial to soil organisms. But more detailed experiments are needed in future to understand impact of various agriculture practices of transgenic plants on functions of soil microbes.

Timms-Wilson [82] refer plants rhizosphere as an 'environmental hotspot' for gene transfer. The horizontal transfer of genes takes place in nature between organisms of different species (*Figure 2.*). It has been suggested that exchange of gene between transgenic plants and soil microorganisms in belowground ecosystem may take place, which can cause structural and functional alterations in soil microorganisms [7, 27]. The plant roots exudate DNA, so transgenic plant DNA is available to soil microoganisms. The persistence of plant DNA in the soil is dependent on abiotic and biotic factors, the content and type of clay minerals and the presence of DNAase enzyme in the soil [8, 83]. Through natural transformation method soil organisms may be transformed by free DNA, released from decomposing plant tissue and stabilized on soil particles. But for natural transformation to occur in a soil environment, free DNA and competent bacteria in the soil need to be in close vicinity [76]. Natural transformation is one of the methods that may allow the dispersal of foreign transgenes, such as antibiotic resistance markers, to native soil bacteria [59, 60,63]. Widmer [16, 83] quantified the antibiotic resistance marker gene persistence in the field, and reported that marker genes from tobacco (*Nicotiana tabacum* L.) and potato (*Solanum tuberosum* L.) were detectable in soil for 77 and 137 days, respectively. Gebhard and Smalla [35] in Germany also reported transfer of transgenic DNA from the transgenic sugar beet plant debris to bacteria in the soil The kanamycin resistance marker gene was transferred to the soil bacterium *Acinetobacter* in an experiment using DNA that was extracted from homogenized plant leaf from a range of transgenic plants, including potato, tobacco, sugar beet, oil-seed rape and tomato [18].

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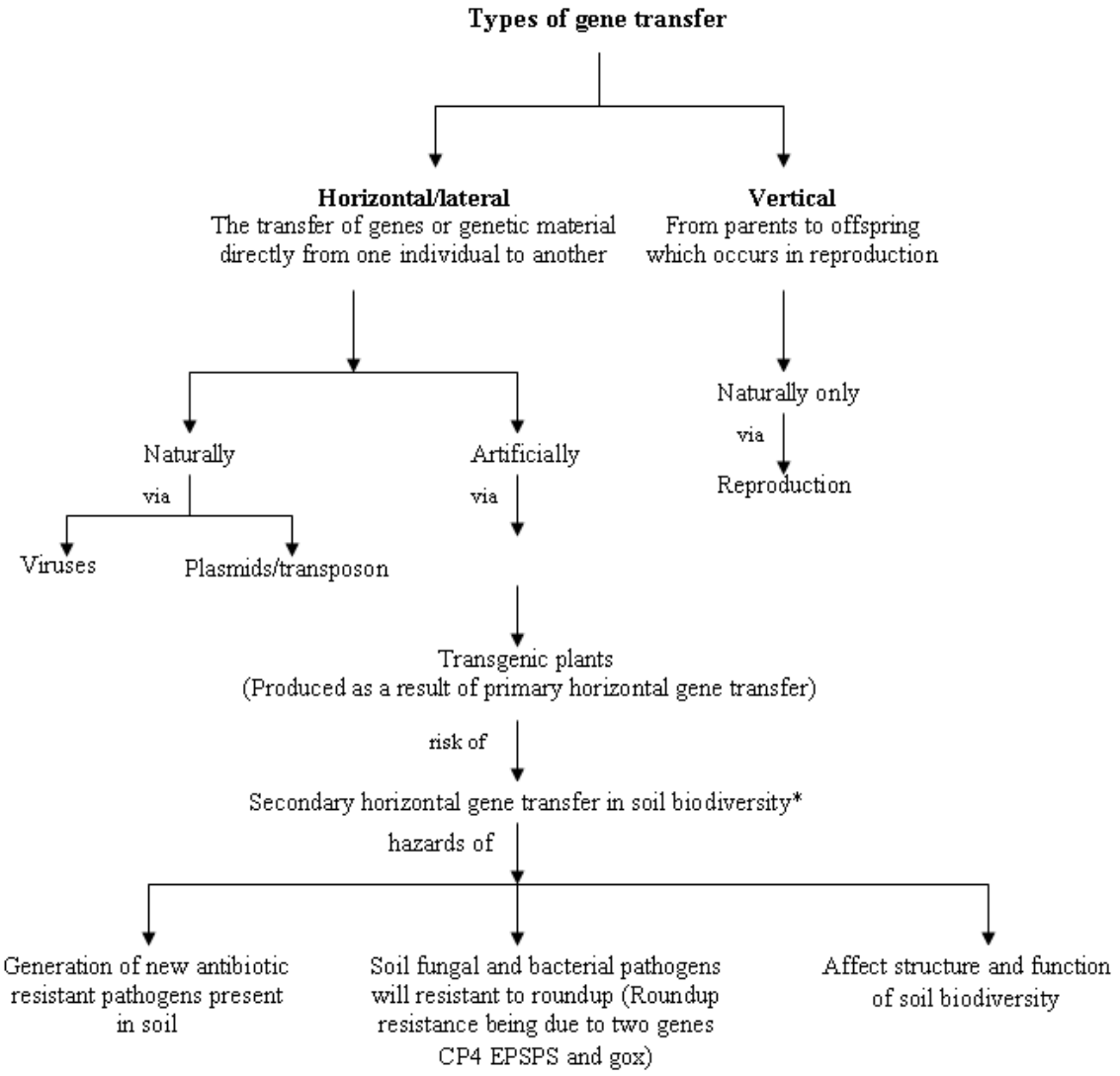

*Secondary horizontal transfer of transgenes and antibiotic resistant marker genes from transgenic crop plants into soil bacteria and fungi have been documented in the laboratory.

Figure 2. *Gene transfer in soil ecosystem*

Major issue of concern from gene exchange is transfer of antibiotic resistance genes to pathogenic microbes present in the soil, rendering them resistant to treatment with such antibiotics Another potential complication might occur when uptake of *Bt* toxin genes (released from Bt crops in soil) by soil microbes. If this occurs, this might lead to more *Bt* toxin production in the soil, to an extent that may be harmful to soil invertebrates that contribute to soil fertility. Horizontal transfer of genes between soil microorganisms and transgenic plants may cause changes or disturbances in the functioning of the microorganisms that indirectly affect that soil ecology and fertility.

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Future framework to assess impact of transgenic plants on soil ecosystem

After reviewing the reports of the studies carried out to assess impact of transgenic plants on soil ecosystem, we observe - (i) results of different studies contradict each other (ii) major gaps in information and baseline data on interactions of transgenic and living and nonliving components of soil still exist. All this at last leads us towards a state of confusion only, which is due to lack of universal schematic approach based framework for assessing the ecological risks and benefits of transgenic plants on soil. The universal framework is imperative and must be based on holistic approach, which will directly reflect response of soil ecosystems to transgenic plants. Transgenic plants impact-monitoring concepts needs to integrate available impact assessment data, environment monitoring networks baseline data, threshold values for impacts with future impact assessment studies on transgenic plants. Future impact assessment studied must be long term and should be carried out prior as well as post release of transgenic crops. As long term monitoring only truly represents slowly changing variables such as biodiversity or accumulation of transgenic crops product in soil. In the integrated framework changes in different soil ecological variables (physical, chemical and biological) due to transgenic plants should be prioritized. As integrated prioritization of impact assessment approach is not only critical in creating reliable monitoring data, the methodology also helps in fast decision making at advance stages of monitoring. The components of future impact assessment framework should be –

(i) Monitoring of retention time or half life of transgenic crops products in soil, if the retention time of transgenic products in soil is very less than they don't have any affect on soil and its diversity, however if retention time is high its its negative or positive impacts should be monitored in different agro climatic zones soil ecosystem.

(ii) Monitoring of changes in spatially and temporally distributed soil ecological variables due to transgenic crops

(iii) Integrated agro-environmental and transgenic plants monitoring network

(iv) Within transgenic plants monitoring network, Inhibition or stimulation of activity of specific microorganism or its community due to differences in the amount and composition of root exudates released from transgenic plants should be observed in different agro environments

(v) Impact of various agriculture practices of transgenic crops agro ecosystems on soil diversity and processes and,

(vi) Monitoring of horizontal gene transfer between transgenic crop and soil diversity at commercialized transgenic crops cultivation sites field

(vii) Changes in functions of microbial populations of soil community due to rare events i.e.,horizontal gene transfer.

Capacity of soil to carry out all its functions sustainably is defined as soil quality [42]. Quality of soil is assessed through the physical, chemical and biological parameters of soil. The physical parameters are -bulk density, texture, infiltration, water holding capacity, aggregation, top soil depth, chemical parameters includes - pH, Organic matter content, nitrogen content in different forms, salinity, nutrient availability and biological parameters are - microbial biomass, shift in trophic level and diversity, soil communities and soil respiration rate etc. But till now integrated studies on impact of transgenic plants on all soil quality indicators are almost lacking. In Future impact assessment studies for assessing the impact of transgenic plants on soil quality of

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diverse agro ecosystems various soil parameters should be monitored to extract real picture.

Any alteration in structure and function of biological representative of soil i.e., either a single species or whole community of a species in transgenic agro ecosystem will represent impact of transgenic crops. Important functions and fertility of good soils are regulated by some dominant microbial species. The focus of the future studied should be on monitoring structure and function of microorganisms or their communities, whose demise is expected to result in the loss of a particular soil function and are therefore indicative of any positive or negative impact of transgenic crops on the soil ecosystem [10, 44]. The impact of transgenic plants on soil can also be observed by monitoring biological baseline components such as keystone species along with abiotic indicators as these exhibit high variability in their functioning within different soils. In addition, the specific ecosystem should be considered and the study should be performed to account for impacts on the key players in that ecosystem. This would mean that if *Pseudomonas* species, for example, were particularly important in a specific ecosystem then their populations and processes mediated by them would be evaluated for that specific ecosystem over the course of the study. Selection of keystone indicators should be done on the basis of their agronomic relevance, ecological significance and responsiveness to perturbations, plus the availability of practical assay methods.

It is recognized that, in addition to the use of keystone indicators, there is a need for broader analyses of the impacts on the soil microbial and faunal communities to improve sensitivity and, importantly, to improve detection of unforeseen effects [46]. Such monitoring requires a combination of measures, each responding to different types of change, for example monitoring the biomass, activity and diversity of the microbial and faunal communities. Monitoring of community size of soil microorganisms such as nematode and collembola, substrate utilization pattern as well as composition of rhizosphere bacterial communities and the metabolic fingerprints of the microbial community in the transgenic crops fields soil will also indicate impacts of transgenic plants on soil ecosystem and will helps us in deciding whether transgenic plants are really an option for sustainable agriculture practices or they itself affecting the sustainability of soil ecosystem

Agriculture practices especially tillage and irrigation plays an important role in enhancing or inhibiting any adverse or positive effect of plants on soil microorganisms. In future studies various agriculture management practices associated with transgenic crops should also be monitored for any influence on the activity of soil microbial communities. As agriculture practices can control the exposure or contact of soil microbial communities with transgenic crop biomass. Horizontal gene transfer between transgenic crop and native soil biodiversity of an agroecosystem is also an important aspect which require regular monitoring through modern techniques

A framework (*Fig. 3*) based on above discussed points will redefine the transgenic plants impact assessment process and ensure more reliable results. This will also help researchers in thorough understanding of impact of transgenic plants on structure and function of soil microbes. Since only a small portion of soil microbial populations can be cultured and identified using standard analytical methods. Combining techniques, especially different types of methods, will in almost all cases lead to more accurate and well-balanced view of the soil system (*Table 2*).

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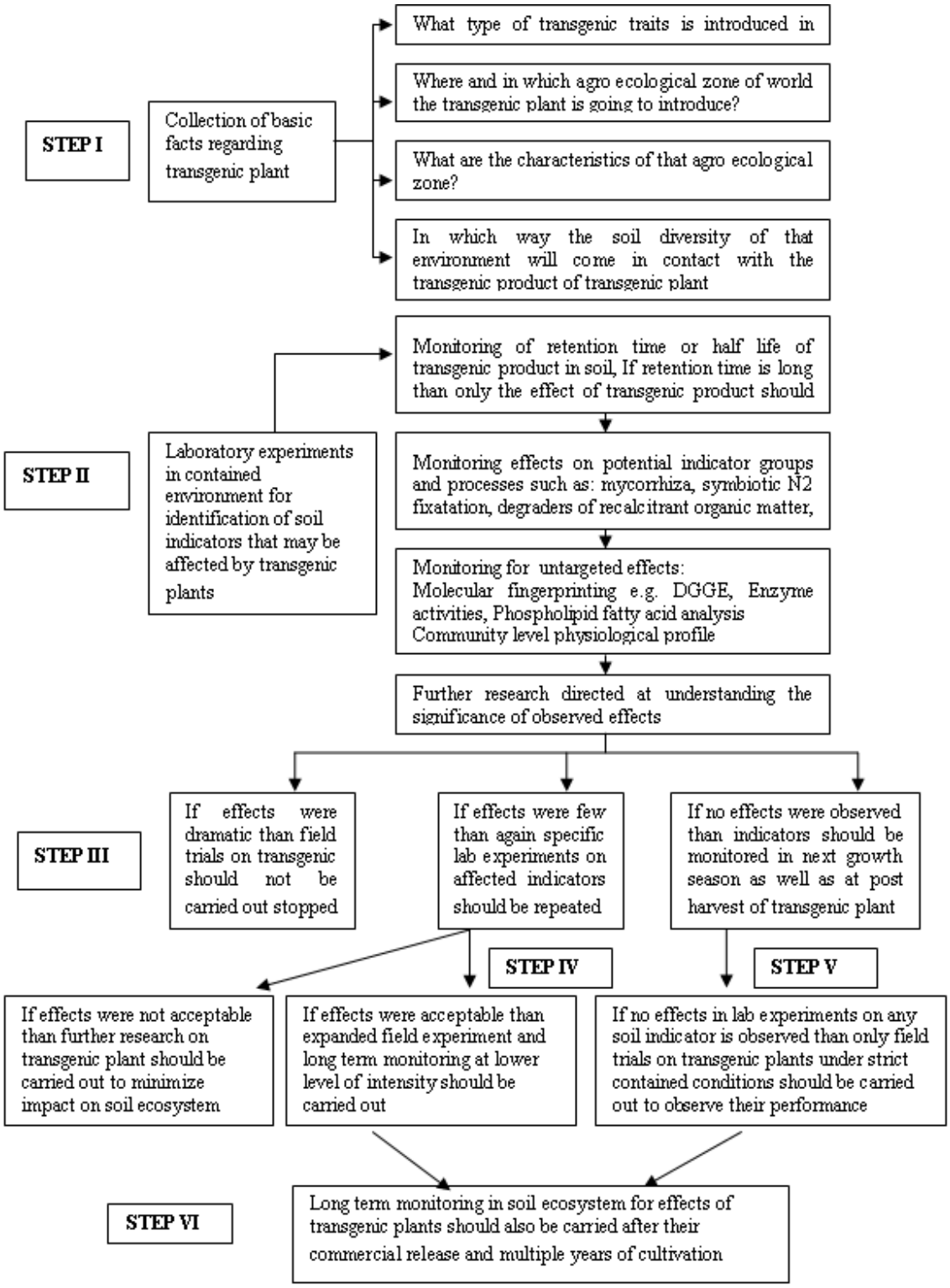

Figure 3. *Schematic approach outline for impact assessment of transgenic plants on soil ecosystem and functions*

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Table 2. *Few examples of horizontal gene transfer in soil ecosystem*

Type of study	Transgenic plants	Gene for transgenic trait	Transferred to	Reference
Lab experiment	Potato, tobacco, canola and tomato	nptII (kanamycin resistance) genes	*Acinetobacter* Soil bacteria	[18]
Lab experiment	Sugar beet	(kanamycin resistance) genes	*Acinetobacter* Soil bacteria	[34]
Lab experiment	Maize	bla gene encoding TEM-1 L-lactamase	*E.coli* bacteria in the saliva and rumen fluid of adult sheeps	[24]

Conclusion

After reviewing the literature on interactions of transgenic plants with soil components we understood that, though lab and field reports on such interactions are very limited, but they have showed that the transgenic plants and their transgene products directly or indirectly leaving their footprints on soil ecosystem which in long term may affects the structure and functioning of soil ecosystem. However due to transient behaviour of impacts and lack of systematic integrated experimental approach in above discussed studies it is very difficult to establish that those changes in soil ecology are solely due to transgenic plants. Because of the exponentially increasing land area under transgenic crops cultivation in last few years, long-term risk assessment studies based on integrated approach are urgently needed in diverse agro-ecological zones of world for filling information gap as well as to establish the net direct and indirect effects of transgenic crops and their associated management practices on soil ecosystem and biodiversity. Environment plays an important role in creating variability in impacts of transgenic plants on soil ecosystem. So, in integrated approach framework environmental monitoring component should be incorporated to generate baseline data on impacts of transgenic plants on different dimensions of soil ecology from various agro ecological zones of world where commercial cultivation of transgenic crops has been allowed. As some studies have shown that transgenic plants may affect biogeochemical cycling in soil ecosystem in long run, which may affect nutrient cycling in hydrosphere and atmosphere also, as all are interlinked. Transgenic crops have immense potential to provide economic and environmental benefit, but few reports which have shown negative impacts of transgenic plants on soil ecosystem have created a doubt on benefits of transgenic crops. So to clarify those doubts and to say anything conclusively, more long term experimental studies which should monitor each and every aspect of soil ecology are urgently needed in each and every nation which have allowed commercial cultivation of transgenic crops

Acknowledgements. The authors are thankful to those who have contributed to the literature on this subject, whose papers have been cited. Special thanks are extended to our respective institutes where we are working.

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USE OF MICROBES FROM SEABIRD FAECES TO EVALUATE HEAVY METAL CONTAMINATION IN ANTARCTIC REGION

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(Received 31st January 2007 ; accepted 24th February 2008)

Abstract. This study evaluates the levels of heavy metal pollution in Antarctic marine ecosystem by monitoring gut microbial flora of seabird skua (*Catharacta* spp). Aerobic and anaerobic bacteria isolated from fresh faecal sample of skua were tested for their heavy metal tolerance and antibiotic resistance pattern. More than 50% isolates were able to tolerate 200 g/ml concentration of lead (Pb), chromium (Cr), zinc (Zn) and nickel (Ni) and >80% isolates showed resistance towards Bacitracin. More than 50% isolates were resistance to multiple antibiotics (MAR) and the the traits were found to be plasmid-borne in nature. The elevated rate of tolerance to the heavy metals reflects an adaptive response to the toxic metals present in marine environment.

Keywords: *Antarctica, skua, gut microflora, heavy metals*

Introduction

The largest and diverse ecosystem on the globe, Antarctica is considered as a major biotic province. Antarctica ecosystems are comprised of marine, freshwater, ice and terrestrial habitats. Besides microbial population, a number of bird species also inhabit Antarctica, which includes polar skuas (*Catharacta* spp). Pollutant level determination in polar skuas (*Catharacta* spp) are interesting, as this species has one of the longest migration of any birds, taking it through polluted wastes of the Northern Hemisphere (Devillers, 1977) as far north as the Grand Banks of Newfoundland (Veit, 1978) and Greenland (Fullager, 1976). Seabirds, which are top predators, are predominantly studied for monitoring changes in lower trophic levels of the marine food chain (Bourne, 1976; Furness and Monaghan; 1987). Because seabirds are conspicuous creatures, they are a suitable choice to play a role as sentinel organisms. Seabirds can range over huge areas of the ocean when feeding or migrating, and many accumulate high concentrations of anthropogenic pollutants while foraging during these flights (Risebrough & Carmignani, 1972; Bull et al, 1977; Furness and Hutton, 1979, 1980; Gardner et al, 1985). Some pollutants are characteristically lipid-soluble but have low water solubility such as organo-chlorines and organo-metallic compounds. Thus, seabirds are appropriate as monitors of food chain exposure to lipid soluble pollutants (Furness, 1993).

The seabirds, skua (*Catharacta* spp.) are scavengers in nature but now they have made their foster home in Antarctica. These birds visit Antarctica during summer season and migrate towards Arctic Ocean during winters. *Catharacta* spp are top predators feeding on fishes and other seabirds. They make their nests around places of human habitation. Being migratory and top predators in marine ecosystem, skuas are ideal species through which to monitor trends in oceanic pollution (Furness, 1987). .Since microbial flora of any habitat indicates the environmental quality status and the exploitation or pollution levels of that particular area this study was attempted with a

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view to assess the pollution levels of Antarctica marine ecosystems by analysing the gut microbial flora of an Antarctic seabird skua (*Catharacta* spp).

Materials and methods

Sampling

Sampling was performed during the mid –summer period (during 18th Indian Expedition to Antarctica, January, 1999). Stool sample from Antarctic skua was collected from the vicinity of Indian station "Maitri" of the Schirmacher Oasis (Queen Maud Land) which is situated in the geographical coordinates of 70º 45' 12" S and 11º 46' E.

Flying individual was tracked using 10x42 binoculars and fresh fecal matter of the skua delivered on a rock was collected aseptically in a sterile container and subjected for bacteriological analysis immediately

Microbiological analysis

Faecal sample was homogenized thoroughly with sterile phosphate buffer saline (pH 7.0) for the isolation of bacteria. Sample was appropriately diluted and plated by the standard spread plate method (APHA, 1992) on plate count agar and anaerobic agar (Hi-Media Ind. Pvt. Ltd.) plates. The inoculated plates were incubated aerobically and anaerobically respectively at room temperature (24°C) for 24 hours. After appropriate incubation, clones representing different colony types were purified on Mac Conkey agar plates.

Characterization of bacterial isolates

Twenty-two colonies purified on the basis of morphological differences were subjected to different morphological and biochemical tests such as gram staining, oxidase, catalase, TSI, etc and were characterized according to Cowan and Steel (1974).

Evaluation of heavy metal tolerance

Tolerance of faecal bacteria to various heavy metals was determined by agar dilution method (Cervantes et al., 1986). In agar dilution method, plates were prepared freshly by supplementing agar medium with metal salts to give a final cationic concentration in µg/ml of 50,100, 200, & 400 for Cd^{2+}, Cu^{2+}, Zn^{2+} Ni^{2+} Pb^{2+} As^{2+} Cr^{2+} and 50 & 100 for Hg^{2+}. The compounds used were $Cd(NO_3)_2$, $CuCl_3$, $ZnCl_2$, $Ni(NO_3)_2$, $(CH_3C00)_2Pb$, As_2O_3, $K_2Cr_20_7$, and $HgCl_2$. Plate were inoculated and incubated aerobically & anaerobically at room temperature (24 ± 2^0C) for 24 hours. The minimum concentration of metals inhibiting complete growth was taken as minimal inhibitory concentration (MIC).

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Determination of antibiotic resistance

Susceptibility to different antibiotics was determined by Disc Diffusion method (Bauer et al., 1966). For determining antibiotic resistance, antibiotic impregnated discs were plated on Mueller-Hinton agar (Hi-Media Ind. Pvt. Ltd) plates that had been seeded with culture. The plates were incubated aerobically and anaerobically at room temperature ((24 ± 2^0C)) for 3-4 days. The isolates were classified as resistant or susceptible following the standard disc sensitivity testing method (Difco, 1884). Discs containing the following antibiotics µg/discs were tested; ampicillin (10), gentamycin (10); vancomycin (30), Tetracycline (30), Polymixin-B (50); chloramphenicol (30); Bacitracin (10); Streptomycin (25); Kanamycin (30), Neomycin (30), Carbenicillin (100); Nalidixic acid (30); cephaloridine (30), Co-trimaxazole (25) and Colistin (25).

Plasmid curing: Spontaneous loss

A total of eight isolates- six aerobic and two anaerobic, resistant to multiple antibiotics were selected for observing plasmid curing and spontaneous loss of resistance. Test organisms to be cured were inoculated in peptone water and were brought up to their logarithmic growth phase. Plasmid curing was observed by the application of curing agent, acridine orange. Tubes containing 10 ml peptone water were supplemented with 20µg/ml acridive orange and were inoculated with 0.1 ml of over night grown broth culture and incubated at 37°C for 24 hours. Loop-full of inoculum was streaked on Mac Conkey agar plates to obtain single colony isolates having stress due to curing agent. After overnight incubation at 37°C, resulting colonies were again tested for loss of heavy metals and antibiotic resistance for which the wild strain was found to be resistant. To determine spontaneous loss of plasmid, cultures were grown in peptone water tubes and transferred at regular intervals and the procedure described above was repeated.

Results

Isolation and characterization results

Isolation of bacteria yielded a total of twenty-two (22) pure colonies, among which six colonies were anaerobic in nature. The isolated organisms were found to be the members of family Enterobacteriaceae. Among the aerobic bacteria, 87.5% of the isolates were found to be *Escherichia coli* (S-1, S-2, S-3, S-4, S-5, S-7, S-8, S-9, S-10, S-11, S-12, S-13, & S-16) while 12.5% isolates were characterised as *Citrobacter* (S-15, S-17). Thus altogether *E. coli* was the predominant genera (77.46%) among the total number of isolates followed by *Citrobacter* and *Providencia*, both representing 9% of the total population while *Klebsiella* (4.54%) were the least reported species (*Fig. 1*). The anaerobic bacterial population represented 50% of the isolates as *E. coli* (SA-2, SA-3, & SA-6) followed by *Providencia* (33.33%, SA-4, SA-5) and *Klebsiella* (SA-1) as 16.67% of the total anaerobes (*Fig. 1*).

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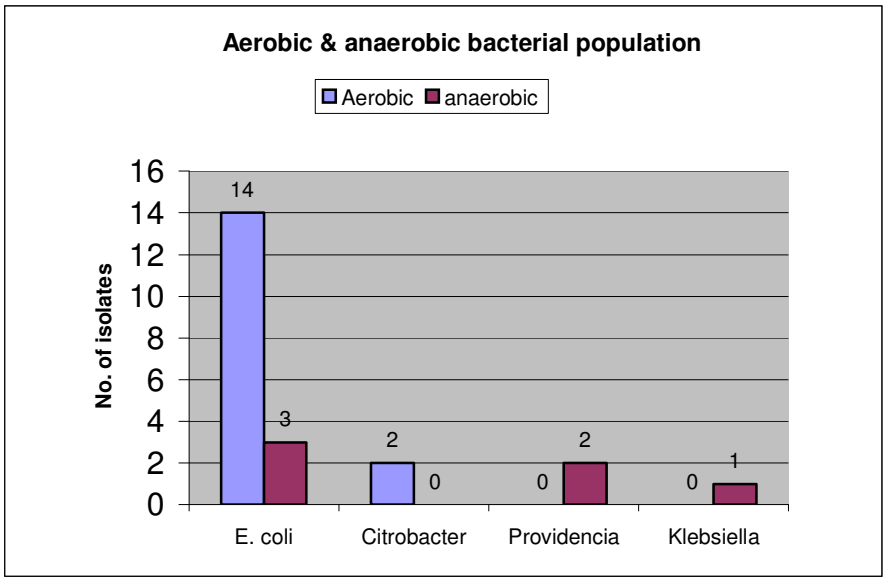

Figure 1. *Types of bacterial strains from skua faeces*

Heavy metal tolerance

The isolated organisms showed various levels of tolerance towards different heavy metals (*Fig. 2*). All the isolates, aerobic as well as anaerobic, were able to tolerate 50 µg/ml concentration of all the heavy metals tested except for mercury (Hg) for which only 68.1% of the isolates showed tolerance.

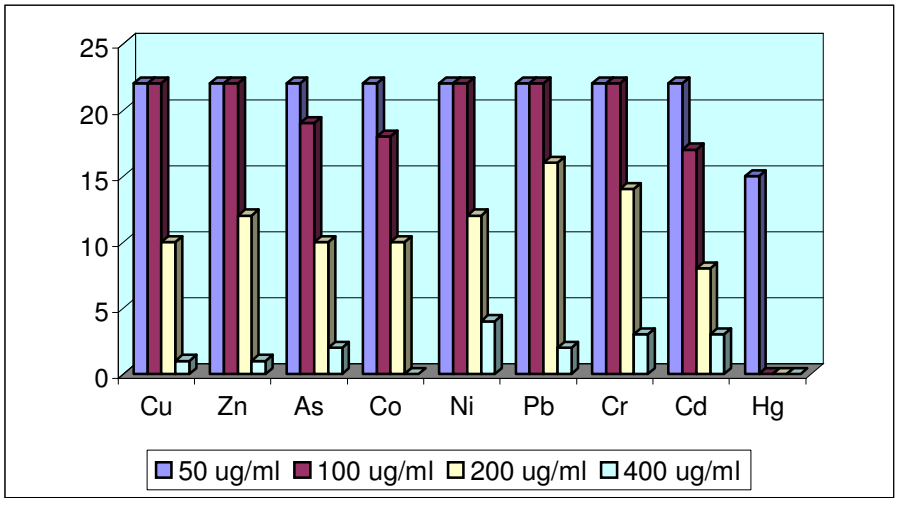

Figure 2. *Heavy metal tolerance among bacterial isolates from skua faeces*

Further, at 100µg/ml concentration of Hg, none isolate was found to be tolerant. However, a tolerance level at this concentration (100 µg/ml) was shown by maximum number of isolates (86.3%) for Arsenic (As), followed by Cobalt (81.8%) and Cadmium (77.2%) isolates. At 200 µg/ml concentrations of heavy metals, maximum tolerance was observed for lead (Pb) shown by 72.7% of the isolates followed by Chromium

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(63.6%), Zinc and Nickel (54.5%), Copper, Arsenic & cobalt (46.4%) and the least for Cadmium (Cd) shown by 36.3% isolates. At the maximum concentration of heavy metal tested i.e.

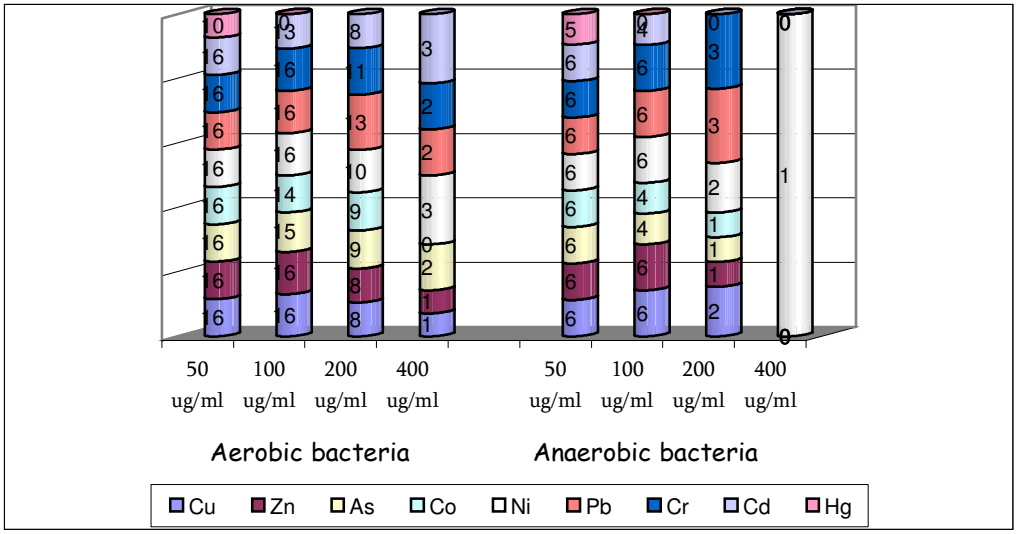

Figure 3. *Comparative analysis of tolerance as shown by aerobic and anaerobic isolates for various heavy metals*

400 µg/ml, highest tolerance was showed by 18.18% of the isolates for Nickel while 13.6% isolates showed tolerance for Chromium and Cadmium respectively. Tolerance for Arsenic and Lead was observed in 9.09% isolates and least tolerance was shown by 4.54% isolates for copper and zinc respectively. None isolate was found tolerant at this level of concentrations for cobalt.

Comparing the tolerance levels of these isolates on their aerobic and anaerobic nature, it was found that aerobic bacterial isolates were more tolerant towards heavy metals as compared to their anaerobic counterparts. A comparative analysis of tolerance as shown by aerobic and anaerobic isolates for various heavy metals is depicted in *Fig. 3*. Tolerance at higher concentration (400 µg/ml) was observed by 16.67% anaerobic isolates only for Nickel while no isolate was tolerant to rest of the heavy metals tested at this concentration.

Among aerobic bacteria none of the isolate showed tolerance for cobalt at 400 µg/ml concentration of heavy metals while maximum tolerance was revealed for Nickel and Cadmium by 18.75% isolates.

Resistance to antibiotic

The microbial isolates from Skua faeces showed a varied pattern of resistance towards different antibiotic (*Fig. 4*). Predominant resistance was observed for bacitracin showed by 81.8% of the isolates. 45% isolates were found to be resistant to both vancomycin and kanamycin while for streptomycin 36.3% of the isolates showed resistance.

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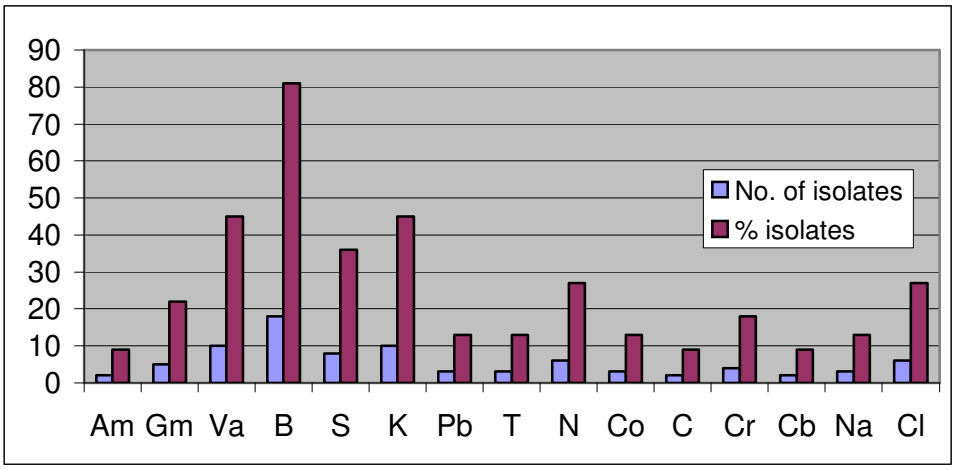

Am: ampicillin, Gm: gentamycin, Va: vancomycin, B: bacitracin, S: streptomycin, K: kanamycin, Pb: polymixin-B, T: tetracycline, N: neomycin, Co: Co-trimaxazole, C: Chloramphenicol, Cr: cephaloridine, Cb: Carbenicillin, Na: Nalidixic acid, Cl: Colistin

Figure 4. *Antibiotic resistance among bacteria from skua faeces*

Resistance for three antibiotics Gentamycin, Neomycin and Colistin was observed in 27.2% of the isolates. 18.1% isolates showed resistance for cephaloridine and 13.6% isolates were found to be resistant for polymixin-B, Tetracycline, Co-trimaxazole and Nalidixic acid. Least resistance was observed for Ampicillin, shown by 9.09% isolates.

As a comparative study for resistance among aerobic and anaerobic isolates aerobic strain were found to be more resistant to various antibiotics (*Fig. 5*).

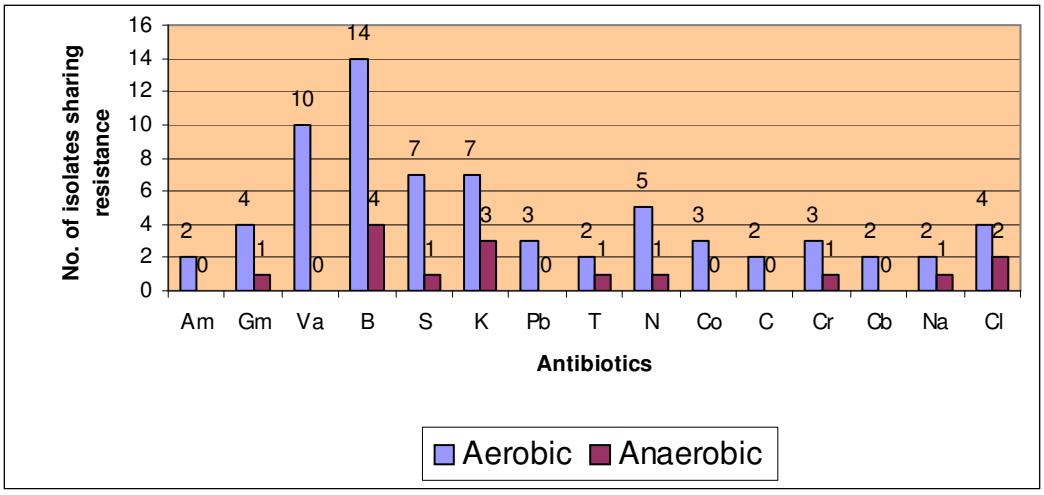

Figure 5. *Comparison between aerobic versus anaerobic isolates sharing antibiotic resistance*

None of the anaerobic isolate was found to be resistant against antibiotics-Ampicillin, vancomycin, Polymixin-B, co-Trimaxazole, chloramphenicol and carbenicillin. Maximum resistance among anaerobic isolates was shown by 66.67% of the isolates for Gentamycin, streptomycin, tetracycline, Neomycin, Cephaloridine and Nalidixic acid.

Among aerobic isolates, predominant resistance was observed by 90% isolates for bacitracin, followed by vancomycin (65%) and streptomycin and Kanamycin (both 45%

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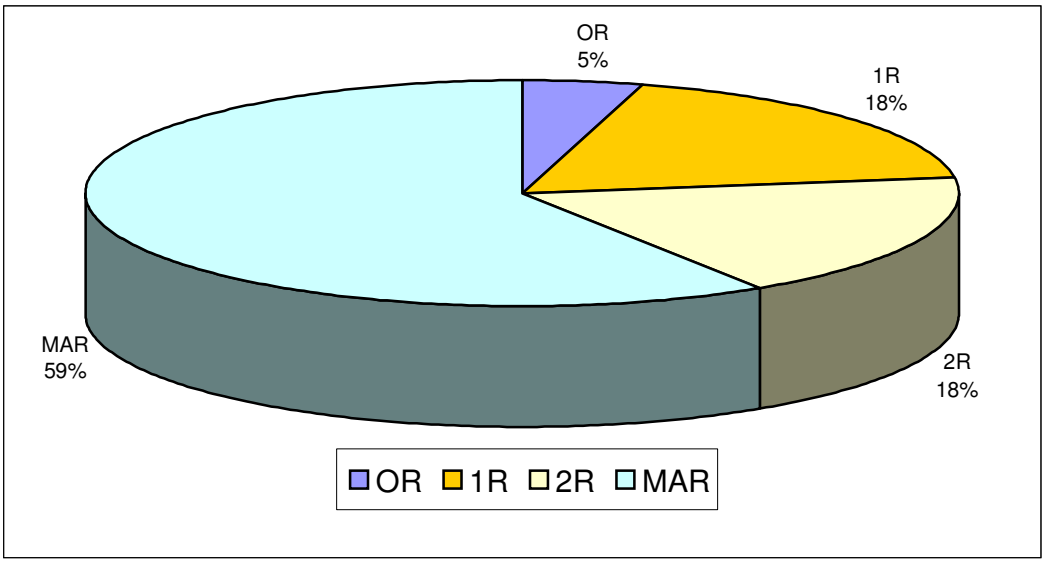
isolates). Least resistance was shown by 12.5% isolates for a number of antibiotics (ampicillin, Tetracycline, chloramphenicol, carbenicillin and Nalidixic acid).

A graphical analysis between the number of isolates resistant to the number of antibiotics is shown in *Fig. 6* which reveals that 59% of the isolates were resistant to multiple antibiotics (MAR) while one aerobic (S-12) isolate was fully susceptible to all the antibiotics tested; not showing resistance towards any antibiotic.



0R: susceptible to all antibiotics, 1R: resistant to one antibiotic, 2R: resistant to two antibiotics, MAR: resistant to multiple antibiotics

Figure 6. *Antibiotic determinants among isolates from skua faeces*

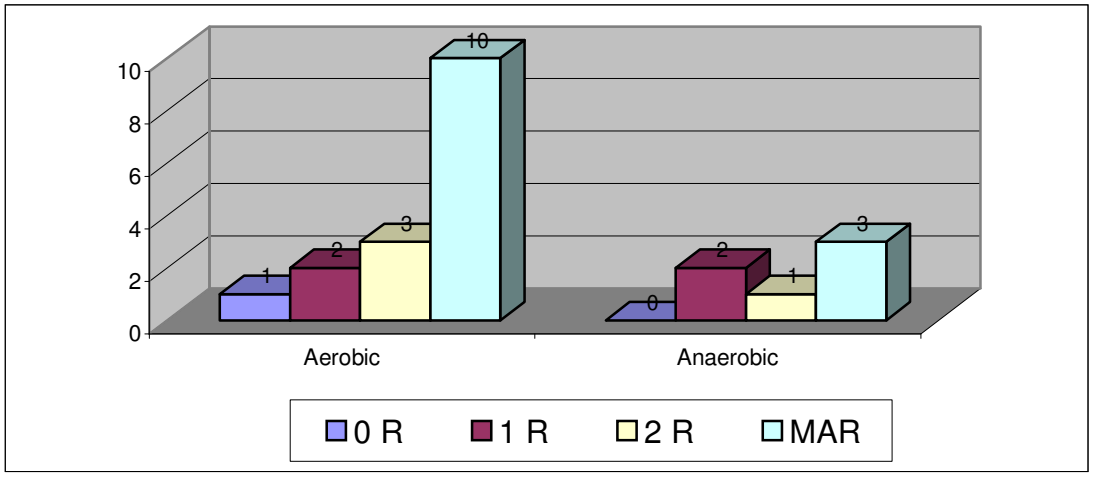


Figure 7. *Comparative graphical analysis of aerobic and anaerobic isolates number resistant to the number of antibiotics.*

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Spontaneous loss of resistance and curing of plasmids

Results of plasmid curing among isolates is depicted in *Table 1.* Most of the colonies showed no loss of resistance due to the presence of curing agents for Cadmium and Nickel. Cent percent curing was observed in one aerobic *E. coli* (S-10) for chromium while one anaerobic isolate of *Providencia* (SA-4) showed 100% loss of tolerance towards Cobalt. Two aerobic isolates of *E. coli* (S-2 and S-10) showed 90-100% curing for copper.

Table 1. *Loss of resistance in various isolates due to stress by curing agent*

Heavy metals	**Resistance %**							
	***E. coli* (S-2)**	***E. coli* (S-9)**	***E. coli* (S-10)**	***E. coli* (S-11)**	***E. coli* (S-13)**	***Citrobacter* (S-15)**	***Klebsiella* (SA-1)**	***Providencia* (SA-4)**
Chromium	30	0	100	78.3	85	0	0	70
Cadmium	20	0	0	0	0	0	0	0
Copper	90	76.6	96.6	0	0	0	0	0
Nickel	61.6	0	43.3	0	0	0	0	0
Cobalt	78.3	71.6	81.6	76.6	73.3	70	88.3	100
Antibiotics								
Streptomycin	88.3	0	33.3	0	0	6.6	23.3	90
Kanamycin	95	88.3	85	66.6	73.3	6.	90	0
Ampicillin	13.3	0	20	0	0	0	0	85
Tetracycline	0	0	43.3	0	0	18.3	60	0
Cephaloridine	35	0	23.3	0	0	0	30	0
Bacitracin	16.6	0	0	0	0	0	0	0
Carbenicillin	36.6	0	0	76.6	0	0	0	0
Nalidixic acid	28.3	0	46.6	83.3	63.3	0	0	0

Among antibiotics 85-95% curing was observed in three aerobic *E. coli* isolates (S-2, S-9, S-10) and one anaerobic *Providencia* isolate (SA-4) for kanamycin while one aerobic *E. coli* (S-2) and one anaerobic *Providencia* (SA-4) showed 85-90% loss of resistance towards streptomycin. The anaerobic isolate, *Providencia* (SA-4) also showed 85% curing for ampicillin while none other isolate was found to be cured for ampicillin. One of the aerobic *E. coli* isolate (S-11), not showing curing for most of the antibiotics tested was found to lose 83.3% of resistance after curing towards Nalidixic acid. Spontaneous loss of resistance was also observed in the same isolates for kanamycin and chromium.

Discussion

Bacterial resistance may be due to the presence of R-plasmid containing genes for both antibiotics & heavy metals (Timoney et al, 1978; Calomiris et al, 1984). Metal tolerance may also be related to the production of capsular polysaccharides usually by Enterobacter group of organisms, which can combine with metals to protect themselves from the toxicity of metals (Bitton & Freihofer, 1978). More often the resistance phenomenon is plasmid borne and transferable in nature resulting its spread among the sensitive aquatic bacteria including coliforms.

The detection of elevated rate of tolerance to heavy metals in the present study as is depicted in *Fig 3* may also reflect an adaptive response to the presence of toxic elements

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in marine environment in addition to the gut-intestine of Skua itself. However, the tolerance to heavy metal toxicity can also be influenced by several environmental factors like constituents of ecosystem, ion interactions, pH, the form and availability of metals to the microbes. Thus, bacterial tolerance to metal toxicity is a significant environmental phenomenon (Sterritt and Lester, 1980; Jones et al, 1986).

The results show 59% bacterial isolates from skua faeces are resistant to more than one antibiotic (MAR) (*Fig. 7*), which may be related to the process of bacterial survival and adaptation, and/or to the inconstancy of selective pressure in the environment. A number of reports confirm the presence of antibiotic resistant bacteria present in the soil samples from Antarctica (Shivaji et al, 1988, 1989a, 1989b).

Plasmid borne nature of resistance is evident by their spontaneous loss and curing by curative agents. Among antibiotics, resistance to Kanamycin was stable in most of the isolates while single isolate reported 80-90% curing for streptomycin and Nalidixic acid respectively indicating their plasmid-linked inheritance. Chromium and cobalt heavy metals also indicate their plasmid-mediated inheritance as 70-100% curing was observed by most of the isolates for these two heavy metals.

Ray et al, 1991 have isolated antibiotic resistant plasmid from Antarctic soil bacteria. Such bacteria may have evolved due to ecological interaction among the antibiotic producing strains and other strains lacking resistance. The bacteria carrying R-plasmids have a greater chance of survival and propagation in natural ecosystems than that of the strains lacking plasmids (Grabow et al. 1975,). Such plasmid carrying bacteria from Antarctica need detailed investigation. As the enzymatic machinery of these microbes is always under stress due to extreme cold condition, they seem to acquire enzymes, which are cold active, heat-labile and freeze-thaw resistant (Shivaji et al, 1994). But, as yet, the exact mechanism by which there microorganisms sense low temperature and adapt to these conditions is unknown. Whether these enzymes linked genes are plasmid-mediated or not will reflect for a new avenue for survival among psychrotrophs through psychrophilic enzymatic machinery.

The incidence of plasmids in bacteria is reported to be greater in polluted compared to clearer sites (Hada & Sizemore, 1981). Hence, in Antarctica, especially in those regions free of human intervention, the frequency of plasmids should be low. Our studies detected plasmids in eight and of twenty-two bacterial isolates indicating the lower artificial selection pressure due to human interference in Antarctica.

Earlier research on heavy metal and organochlorine pollution in Antarctic wildlife identified the brown skua (*Catharacta lonnbergi*) and the south polar skua (*Catharacta maccormicki*) as having some of the highest pollutant levels of all birds sampled (Norheim et al, 1982; Risebrongh and Carmignani, 1972; Gardner et al, 1985). Hence, it is possible that these species may be the marine equivalents of certain raptors, which show light pollutant loads through exposure to polluted environments when migrating and on wintering grounds (Cade and Fyfe, 1970; Peakall et al., 1976).

The present study sheds light on the pollution level in the Antarctic marine as well as terrestrial ecosystem and through the isolation of plasmid-bone characters provides better biotechnological prospects for survival in the fluctuating extreme external environment.

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Acknowledgements. PWR wish to thank Director, National Centre for Antarctic and Ocean Research (Department of Ocean Development), Vasco-de-Gamma, Goa, for providing an opportunity to be a summer member of 18th Indian Scientific Expedition to Antarctica. Financial support by Council of Scientific and Industrial Research (CSIR) is gratefully acknowledged. The authors are thankful to CSIR, Govt of India for financial assistance.

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A STUDY ON GROWTH CO-EFFICIENT AND RELATIVE CONDITION FACTOR OF THE MAJOR CARP (*CATLA CATLA)* IN TWO LAKES DIFFERING IN WATER QUALITY

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(Received 26th July 2006 ; accepted 16th January 2008)

Abstract. Physico-chemical water quality parameters viz. temperature, pH, alkalinity, turbidity, total solids, DO, BOD, nitrite, nitrate, phosphate, ammonia, and hydrogen sulfide coupled with diversity and density of plankton were studied during different seasons (monsoon, winter, summer) for two consecutive calendar years in two lakes to find out whether differences in water quality are reflected in growth co-efficient and relative condition factor of the major carp, *Catla catla*. Significantly higher levels of nutrients (phosphate, nitrate, nitrite) and ammonia, low plankton diversity, and significantly higher density of cyanophyceae were found in Yennehole lake (YL) compared to Belikere lake (BL). In addition, desmids were conspicuous by their absence in YL in contrast to their presence in BL. Concomitantly, growth factor (b) and relative condition factor (Kn) of *C.catla* were < 3 and < 1 respectively in YL whereas they were close to 3 (or > 3) and >1 respectively in BL in all the seasons in during the study period. The contrasting b and Kn values of the same species in two lakes differing in water quality provide an evidence that deterioration in water quality though not enough to cause death of fish, does adversely affect growth and well being of fish.
Keywords*: Fish growth, water quality, Catla catla, relative condition factor, Algal bloom.*

Introduction

There are good numbers of studies on seasonal variation in water quality of lakes (Banerjea 1967, Munawar 1970, Boyd 1979, Singh & Rai 1984, Ansari & Prakesh 2000, Sharma and Rathore 2000, Khabade et al. 2002, Datta et al. 2005, Sukamarn & Das 2005). On the other hand there are studies on growth and well being of fish in water bodies (Le'Cren 1951, Jhingran 1952, 1968, Tesch 1968, Doha & Dewan 1967, Botros 1970, Bashirulla & Kader 1970, Siddique 1977, Quadri & Mir 1980, Olatunde 1983, Nwadairo 1985, Rekhow 1987, Prinsloo & Schoobee 1987, Staggis & Otis 1996, Sharma & Sinha 2000, Patgiri et al. 2001, Solanki et al. 2004, Boniamin laskar et al. 2005). However these studies did not investigate whether prevailing conditions were congenial for well being of fish. The merological approach for the studies on water bodies might not be useful in assessing suitability of water bodies for fish culture, whereas hololological approach with emphasis on fish growth, water quality, and plankton diversity and density might be useful. Hence, there is a need to investigate variation in fish growth parameters along with physico-chemical and biological water quality parameters, to find out whether variation in growth and well being of fish are influenced by fluctuations in water quality. Studies on these lines are essential to assess suitability of water bodies for fish culture. In the present study we have compared growth co-efficient and relative condition factor of the major carp, *Catla catla* in two

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during different seasons in two perennial lakes to find out whether difference if any in water quality affects growth and relative condition factor.

Materials and methods

The present study was conducted on two perennial rain fed lakes viz. Yennehole lake (YL) (120° 12' 22" N and 760° 31' 12" E 717 mts MSL) and Bilikere lake (BL) (12° 19' 47" N and 76° 27' 45" E 810.46 mts MSL). Both the lakes are being utilized for fish culture since 10 – 15 years. YL is situated in southwest region of Mysore City and spread over about 200 acres and has depth of about 3 – 4 mts. It is mainly used for culture of commercially important major carps. BL has an area of 32.5 acres and depth about 3 to 3.5 mts, which is utilized for irrigation, fish culture and domestic purposes.

The length – weight relationship of the major carp *C. catla*, was studied for two calendar years i.e. January 2002 to December 2003. During the study period length and weight of 15 specimen from each lake were recorded separately at monthly intervals. Later the data were pooled season wise (summer, monsoon and winter) to compute average for each season. The total length and the body weight were recorded immediately after the collection with the help of measuring board and weighing balance. The values of length and weight were determined to the nearest mm and mg respectively. The length- weight relationship was determined using the parabolic equation

(LeCren 1951), $W= a L^b$ (where W= weight of the fish, a= Multiplying constant, b= exponent of length , the growth factor and L= length of fish) for a given season. .

The correlation co-efficient 'r'was calculated by using following formula (Haynes, 1982).

$$r = \Sigma XY - X \Sigma Y / \sqrt{[(\Sigma X^2 - X \Sigma X) (\Sigma Y^2 - Y \Sigma Y)]}$$

The relative condition factor (Kn) was computed by using formula (Le Cren, 1951),

$K_{n.}= W^o / W^c$, Wherein W^o = Observed weight of the fish, and W^c = Calculated weight of the

fish.

Water samples were collected from three sampling sites at monthly intervals from both lakes. Samples were collected between 08 00 to 10 00. Temperature, pH and DO were recorded on spot using portable kit. Total alkalinity, turbidity, TSS, BOD, hydrogen sulphide, phosphate nitrite and nitrate and ammonia were determined separately for all the samples in the laboratory by employing methods described in standard methods (APHA 1995). Arithmetic mean of each parameter computed considering values of three sampling sites. Monthly mean values were pooled to obtain season wise average.

Water samples for studying of phytoplankton and zooplankton diversity and density were collected simultaneously using nets of mesh size 20 µm and 45µm respectively. One hundred liter of sub surface water was filtered through the nets and organisms were collected in glass bottles tied to the bottom of the net. Zooplankton were immediately transferred to specimen tubes with 4% formalin, whereas phytoplankton to Lugol's solution for later microscopic observation. Plankton counts were made in laboratory using Sedgewick rafter counting cells (Welch 1952). Values were computed and expressed in units/ liter. Phytoplankton were identified following keys of Desikachary

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(1959), Gonzalvis (1981) and Prescot,(1982). Zooplankton were identified following description of Edmondson (1959), Battish (1992), and Dhanapathi (2000).

Results

Growth co-efficient and relative condition factor

The growth co-efficient (b) was less than 3 in all the seasons in both the years in YL whereas in BL it was close to 3 or > 3 in all seasons except monsoon, 2002. The correlation coefficient (r) ranged from 0.6004 (summer 2003) to 0.9251 (monsoon 2003) during different seasons of study period (two years) in YL whereas it ranged from 0.8376 (winter 2002) to 0.9248 (monsoon 2003) in BL. The values of correlation co-efficient were statistically significant ($p < 0.05$) for all the seasons and for both the lakes.

The relative condition factor (Kn) was < 1 in all the seasons in two years in YL whereas it was 1 or > 1 in all the seasons during same period in BL.

Table 1. *Length-weight relationship of Catla catla in Yennehole lake (YL) and Bilikere lake (BL) during 2002*

Season & Lake	Number of Fish studied	Growth co-efficient (b)	Calculated 'a'	$W=aL^b$	Correlation co-efficient (r)	Relative condition factor (Kn)
YL Monsoon	60	2.61561	3.77477	$3.7747L^{2.61561}$	0.90014	0.87776
BL	60	2.60803	0.12923	$0.12923L^{2.60803}$	0.86684	1.0084
YL Winter	60	2.10089	4.17332	$4.17332L^{2.10089}$	0.90452	0.9612
BL	60	3.02897	0.93407	$0.93407L^{3.02897}$	0.8376	1.0539
YL Summer	60	2.71786	0.09595	$0.09595L^{2.71786}$	0.87046	0.93941
BL	60	3.20223	0.01601	$0.01601L^{3.20223}$	0.85716	1.0350

Table 2. *Length-weight relationship of Catla catla in Yennehole lake (YL) and Bilikere lake (BL) during 2003*

Season & Lake	Number of Fish studied	Growth co-efficient (b)	Calculated 'a'	$W=aL^b$	Correlation co-efficient (r)	Relative condition factor (Kn)
YL Monsoon	60	2.24267	0.45181	$0.45181L^{2.24267}$	0.92512	0.86503
BL	60	3.30474	0.01697	$0.01697L^{3.30474}$	0.9248	1.0177
YL Winter	60	2.09001	0.67138	$0.67138L^{2.09001}$	0.8742	0.98708
BL	60	3.34729	0.00932	$0.00932L^{3.34729}$	0.9012	1.0134
YL Summer	60	1.52285	5.12968	$5.12968L^{1.52285}$	0.60046	0.8576
BL	60	2.98760	0.04285	$0.04285L^{2.98760}$	0.87426	1.0021

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Physico-chemical water quality parameters

Temperature in both lakes, in both the years of study did not show significant variation amongst different seasons. Further there was no significant variation in water temperature between both the lakes in all the seasons during the study period. The pH showed significant variation in both lakes in 2002, but not in 2003. The pH of BL was significantly lower compared to YL during winter of 2002 and monsoon and summer of 2003. During other seasons of the study period it did not differ significantly. Turbidity did not significantly vary amongst different seasons in YL in both years and in 2003 in BL. However, a significant variation was observed in 2002 in BL. Turbidity was significantly higher in YL compared to BL in all the seasons in both the years.

TSS did not significantly vary in BL in all seasons in both years whereas a seasonal variation was observed during 2003, but not in 2002 in YL. There was no significant difference between TSS of two lakes throughout study period except a significant decrease in BL in monsoon 2003 compared to YL. Total alkalinity did not significantly vary amongst different seasons in both years in YL. However it showed significant seasonal variation in 2002, but not in 2003 in BL. Total alkalinity of BL in winter and summer of both years was significantly lower compared to YL in both years, whereas it did not significantly differ during monsoon of both years.

DO content of YL did not show significant variation amongst different seasons in both years. DO content was significantly lower in winter and summer compared to monsoon in BL during 2002. A similar pattern was seen in 2003, however drop in winter 2003 was not significant compared to monsoon. In both years DO content of BL was significantly lower during winter and summer compared to that of YL whereas it did not differ during monsoon. The BOD did not significantly vary amongst different seasons in both years in YL whereas a significant seasonal variation was observed in 2003 in BL but not in 2002. Excepting monsoon in both years BOD of YL was significantly higher than BL in both years. Hydrogen sulphide content remained unchanged throughout the year in both lakes and it was significantly higher in YL compared to BL in all seasons. Nitrite concentration did not show significant seasonal variation in both years in BL, whereas it showed significant seasonal fluctuation in YL in 2003, but not in 2002. Nitrite concentration of BL was significantly lower in all the seasons compared to YL in both years. Nitrate concentration did not significantly vary amongst different seasons in both lakes in 2002. However in 2003 there was a significant seasonal variation in nitrate concentration in both lakes. The nitrate concentration of YL was significantly higher in all the seasons in both the years compared to BL. Phosphate concentration did not significantly vary amongst different seasons in both lakes in both years, and in all the seasons it was higher in YL compared to BL. In YL ammonia concentration varied significantly amongst different seasons in both years but in BL it did not. During entire study period ammonia concentration was significantly higher in YL compared to BL.

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Table 3. *Seasonal variation in physico-chemical water quality parameters of Yennehole lake (YL) and Belikere lake (BL) during 2002*

	Yennehole Lake			
Parameters	**Monsoon**	**Winter**	**Summer**	**ANOVA F-value**
Temperature (°C)	26.05± 0.71	25.03 ± 1.71	26.37 ± 0.81	0.35 NS
pH	9.01 ± 0.11[a]	9.45± 0.13[b]	9.02± 0.009[a]	5.476 *
Turbidity (NTU)	54.34± 8.07	66.18± 8.62	76.24± 8.67	1.678 NS
TSS	1.26± 0.006	0.67± 0.53	0.60± 0.21	3.293 NS
Total alkalinity	960.37± 60.4	1080.4± 135.7	1165± 110.4	0.926 NS
DO	18.56± 2.87	15.23± 0.18	17.36± 3.57	1.025 NS
BOD	47.14± 12.97	24.71± 1.81	41.01± 12.01	1.277 NS
H_2S	5.29± 0.39	2.58± 023	4.42± 1.38	2.698 NS
Nitrite	0.70± 0.17	0.88± 0.11	0.47± 0.11	2.124 NS
Nitrate	1.0± 0.00	0.94± 0.1	0.73± 0.008	2.544 NS
Phosphate	1.87± 0.18	1.44± 0.16	1.55± 0.1	1.942 NS
Ammonia (µg/L)	286.49± 82.03[a]	171.30± 19.6[b]	235.24± 52.24[a]	8.772 *
	Bilikere Lake			
Temperature (°C)	26.81 ± 1.43	25.34 ± 1.05	26.81±1.43	0.399 NS
pH	9.09± 0.009[a]	8.52± 0.14[b]	8.99± 0.009[a]	24.961 *
Turbidity (NTU)	30.98± 3.09[a]	17.28± 6.34[a,b]	9.32± 2.29[b]	6.536 *
TSS	0.72± 0.003	0.45± 0.34	0.36± 0.008	2.823 NS
Total alkalinity	899.16± 15.62[a]	426.6± 67.16[b]	859.99± 57.84[a]	6.391 *
DO	14.71± 2.33[a]	9.48± 0.76[b]	9.62± 0.94[b]	5.034 *
BOD	37.30± 12.67	14.43± 1.70	14.34± 1.38	3.172 NS
H_2S	1.01± 0.20	0.56± 0.1	0.76± 0.16	1.994 NS
Nitrite	0.004± 0.003	0.007± 0.006	0.0006± 0	0.606 NS
Nitrate	0.49± 0.16	0.53± 0.12	0.39± 0.12	0.304 NS
Phosphate	0.42± 0.25	0.17± 0	0.007± 0	1.311 NS
Ammonia (µg/L)	39.24± 8.04	30.38± 4.24	28.16± 12.08	0.451 NS

Parameters	**Seasons**		
	Monsoon	**Winter**	**Summer**
Temperature	NS	NS	NS
pH	NS	$P < 0.05$	NS
Turbidity	$P < 0.05$	$P < 0.05$	$P < 0.05$
TSS	NS	NS	NS
Total alkalinity	NS	$P < 0.05$	$P < 0.05$
DO	NS	$P < 0.05$	$P < 0.05$
BOD	NS	$P < 0.05$	$P < 0.05$
Hydrogen sulphide	$P < 0.05$	$P < 0.05$	$P < 0.05$
Nitrite	$P < 0.05$	$P < 0.05$	$P < 0.05$
Nitrate	$P < 0.05$	$P < 0.05$	$P < 0.05$
Phosphate	$P < 0.05$	$P < 0.05$	$P < 0.05$
Ammonia	$P < 0.05$	$P < 0.05$	$P < 0.05$

Note: All parameters other than temp, pH, turbidity and ammonia are mg/L. Mean values of each parameter compared by one way ANOVA followed by Duncan's multiple range test. Values with same superscript letter in different seasons for the given lake (rows) are not significantly different. Whereas those with different superscript letters are significantly ($P<0.05$) different. * $P<0.05$.
Comparison of each parameter of YL and BL in each season as judged by Students t- test (significant if $P < 0.05$)

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Table 4. *Seasonal variation in physico-chemical water quality parameters of two lakes during 2003*

	Yennehole Lake			
Parameters	**Monsoon**	**Winter**	**Summer**	**ANOVA F-value**
Temperature (°C)	26.64± 1.28	25.49± 1.72	26.34± 0.99	0.158 NS
pH	9.14± 0.009	9.29± 0.009	9.35± 0.004	2.481 NS
Turbidity (NTU)	76.33± 9.0	64.88± 7.51	50.77± 14.77	1.400 NS
TSS	0.65 ± 0.009^{a}	0.70 ± 0.12^{a}	0.40 ± 0.004^{b}	5.080 *
Total alkalinity	929.34± 37.13	955.97± 38.17	995.21± 81.48	0.348 NS
DO	12.83± 0.62	15.0± 0.96	10.93± 1.35	3.947 NS
BOD	48.67±8.46	32.49±2.98	44.59±7.11	1.621 NS
H_2S	3.56±0.99	3.02±0.76	3.49±0.72	0.056 NS
Nitrite	0.66 ± 0.19^{a}	1.21 ± 0.12^{b}	0.29 ± 0.009^{a}	10.08 *
Nitrate	$0.89\pm0.008^{a,b}$	1.19 ± 0.13^{b}	0.63 ± 0.008^{a}	7.432 *
Phosphate	1.72± 0.22	1.79± 0.22	1.62± 0.007	0.212 NS
Ammonia (µg/L)	195.83 ± 58.74^{c}	82.66 ± 7.33^{a}	133.33 ± 17.78^{b}	24.48 *
	Bilikere Lake			
Temperature (°C)	27.02± 1.29	26.00± 1.06	27.37± 0.34	0.413 NS
pH	8.98± 0.008	8.94± 0.12	8.88± 0.21	0.510 NS
Turbidity (NTU)	33.04± 6.44	20.97± 6.61	15.9± 2.50	2.540 NS
TSS	0.37± 0.004	0.51± 0.12	0.32± 0.007	1.267 NS
Total alkalinity	519.99± 167.89	415.83± 74.44	429.16± 161.65	0.161 NS
DO	12.25 ± 1.56^{b}	$9.93\pm 0.71^{a,b}$	7.24 ± 0.62^{a}	5.652 *
BOD	37.99 ± 8.81^{b}	16.30 ± 1.88^{a}	$17.54\pm3.20^{a,b}$	5.024 *
H_2S	0.70±0.17	0.78±0.15	0.62±0.10	0.387 NS
Nitrite	0.0008±0.0	0.29±0.15	0.0003±0.0	3.544 NS
Nitrate	0.24 ± 0.10^{a}	0.71 ± 0.11^{b}	0.31 ± 0.005^{a}	7.098 *
Phosphate	0.50± 0.41	0.68± 0.25	0.21± 0.16	0.669 NS
Ammonia (µg/L)	18.90± 4.68	16.46± 5.10	22.58± 6.86	0.300 NS

Parameters	**Seasons**		
	Monsoon	**Winter**	**Summer**
Temperature	NS	NS	NS
pH	P < 0.05	NS	P < 0.05
Turbidity	P < 0.05	P < 0.05	P < 0.05
TSS	P < 0.05	NS	NS
Total alkalinity	NS	P < 0.05	P < 0.05
DO	NS	P < 0.05	P < 0.05
BOD	NS	P < 0.05	P < 0.05
Hydrogen sulphide	P < 0.05	P < 0.05	P < 0.05
Nitrite	P < 0.05	P < 0.05	P < 0.05
Nitrate	P < 0.05	P < 0.05	P < 0.05
Phosphate	P < 0.05	P < 0.05	P < 0.05
Ammonia	P < 0.05	P < 0.05	P < 0.05

Note: All parameters other than temperature, pH, turbidity and ammonia are mg/L. Mean values of each parameter compared by one way ANOVA followed by Duncan's multiple range test. Values with same superscript letter in different seasons for the given lake (rows) are significantly different. Whereas those with different superscript letters are significantly (P<0.05) different. * P<0.05. Comparison of each parameter of YL and BL in each season as judged by Students t- test (significant if P < 0.05)

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Diversity and density of plankton study

Forty species of phytoplankton and 24 species of zooplankton were found in YL whereas 68 species of phytoplankton and 42 species of zooplankton were found in BL. Phytoplankton belonged to five different classes *viz.* cyanophyceae, chlorophyceae, bacillariophyceae, euglenophyceae and desmidiaceae. Zooplabnkton belonged to orders rotifera, copepoda, cladocera and ostracoda.

Phytoplankton population of YL was dominated by cyanophycean members in all the seasons of both years. Summer of 2002 recorded a significant increase in cyanophycean population compared to other two seasons. Same pattern was found in 2003 but the increase was not significant. Cyanophyceae in YL was represented by 10-12 species in different seasons of 2002 and 2003. *Microcystis arruginosa* kutz; was most dominating species among them and it was followed by several species belonging to genera, *Oscillatoria, Anabaena and Nostoc*. They formed a thin bloom on the water surface almost all the seasons of the study period. In BL cyanophycean density showed significant seasonal variation in 2003 but not in 2002. Cynophyte density in BL was significantly lower compared to YL in all the seasons of both the years. Chlorophyceae was third dominant group in YL and their density did not show significant seasonal variation in both the years. Chlorophytes were represented by 6-9 different species during study period. Phytoplankton of BL was dominated by chlorophytes, represented by 16-24 species during different seasons and their density showed significant seasonal variation in 2002, with an increase in density in summer. Similar pattern was found in 2003. Chlorophyceae density of BL did not show significant difference with that of YL in all the seasons in both years, except a significant decrease in monsoon 2002.

Bacillariophyceae were second dominant class in YL and their density did not show significant seasonal variation in both years. Bacillariophytes in YL were represented by 8-12 different species in different seasons of the study period. In BL bacillariophytes showed significant seasonal variation in both years. However, the pattern of variation was not similar in consecutive years. Density of bacillariophytes in YL and BL did not significantly differ in all the seasons, except a significant decrease in BL in monsoon 2002. Density of euglenophytes in YL did not show any significant seasonal variation in both the years and it was represented by 8-9 different species in different seasons of study period. In BL euglenophytes were represented by 6-7 species in different seasons of both the years and showed significant seasonal variation in their density in both years. However pattern of variation was not similar in consecutive years. Euglenophytes density of BL was lower than YL in all the seasons.

Desmids were conspicuous by their absence in YL in both the years whereas in BL desmids population was quite considerable in all the seasons of both the years and showed significant seasonal variation in both years, with high density in monsoon. They were represented by 8 -11 species in different seasons of the study period.

Rotifer density in both lakes showed significant seasonal variation with high density in summer compared to other seasons. In all the seasons during two-year period rotifer density was significantly lower in BL compared to YL. However, rotifers were represented by more number of species in BL than YL. Copepoda in YL were represented by 3-4 species their density showed significant seasonal fluctuation in 2002, but not in 2003. In BL copepod density was significantly increased in winter 2002.

Copepodes were represented by 5-7 species in different seasons in BL and their density in BL during all the seasons of 2002 and in winter of 2003 was significantly lower compared to YL.

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Table 5. *Seasonal variation in plankton density in Yennehole lake and Bilikere lake, 2002*

Plankton / Lake	Monsoon	Winter	Summer	ANOVA F-value
Cyanophyceae YL	29050±4031.23[a] (12)	34025±4219.86[a,b] (12)	40870±1265.66[b] (10)	5.963 (P <0.05)
BL	6425 ±782.49 (09)	9300±1149.63 (07)	9750±1354.31 (08)	2.591 (NS)
Chlorophyceae YL	9500±1666.33 (09)	8000±804.15 (08)	9250±717.05 (08)	0.492 (NS)
BL	6725±601.90[a] (13)	9800±963.50[a,b] (16)	12100±1948.07[b] (16)	5.290 (P <0.05)
Bacillariophyceae YL	10600±1292.92 (09)	9050±494.13 (11)	9300±1068.48 (12)	0.679 (NS)
BL	6200±302.76[a] (10)	11200±721.11[b] (11)	8740±186.84[a,b] (10)	6.018 (P <0.05)
Euglenophyceae YL	9400±355.90 (08)	10600±948.68 (09)	8200±989.10 (08)	2.155 (NS)
BL	7175±661.28[b] (06)	4200±452.76[a,b] (07)	3550±545.43[a] (07)	11.925 (P <0.05)
Desmids YL	NIL	NIL	NIL	NIL
BL	7900±511.53[b]	3800±374.16[a]	4500±254.95[a]	30.921 (P <0.05)
Rotifers YL	643±32.54[a] (10)	613±36.77[a] (12)	858±86.43[b] (11)	5.421 (P < 0.05)
BL	218±26.77[a] (17)	138±15.87[a] (15)	422±68.43[b] (19)	11.382 (P <0.05)
Copepods YL	580±67.56[a] (04)	636±67.42[a] (04)	298±14.86[b] (04)	13.034 (P < 0.05)
BL	171±32.60[a] (07)	292±7.39[b] (06)	168±12.49[a] (07)	12.999 (P <0.05)
Cladocera YL	182±16.87 (04)	210±11.80 (04)	146±12.86 (03)	2.641 (NS)
BL	122±20.89[a] (05)	248±23.62[b] (05)	108±20.86[a] (06)	12.496 (P < 0.05)
Ostracods YL	74±12.36[a] (04)	77±18.96[a] (03)	38±2.86[b] (03)	5.665 (P<0.05)
BL	57±11.56[a] (06)	80±12.94[b] (06)	56±3.16[a] (04)	11.600 (P< 0.05)

Plankton	Seasons		
	Monsoon	Winter	Summer
Cyanophyceae	P < 0.05	P < 0.05	P < 0.05
Chlorophyceae	P < 0.05	NS	NS
Bacillariophyceae	P < 0.05	NS	NS
Euglenophyceae	NS	P < 0.05	P < 0.05
Desmids	il	Nil	Nil
Rotifers	P < 0.05	P < 0.05	P < 0.05
Copepods	P < 0.05	P < 0.05	P < 0.05
Cladocera	NS	NS	NS
Ostracods	NS	NS	NS

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Table 6. *Seasonal variation in plankton density in Yennehole lake and Bilikere lake, 2003*

Plankton (units/lits)		Monsoon	Winter	Summer	ANOVA F-value
Cyanophyceae	YL	31875±4248.79 (13)	34475±4819.98 (12)	45100±6258.06 (10)	2.062 (NS)
	BL	8100±866.02[a] (11)	14250±1374.46[b] (09)	11100±1707.82[a,b] (11)	5.107 (P<0.05)
Chlorophyceae	YL	9800±904.23 (06)	9500±665.83 (09)	10100±854.40 (08)	0.136 (NS)
	BL	9100±782.09[a] (16)	7500±823.60[a,b] (16)	11550±1060.26[b] (16)	5.171 (P<0.05)
Bacillariophyceae	YL	8000±580.22 (09)	10550±1447.12 (10)	9125±604.66 (08)	1.752 (NS)
	BL	7900±491.59[a] (10)	10600±960.03[b] (12)	13800±875.59[c] (13)	13.119 (P <0.05)
Euglenophyceae	YL	8100±1191.63 (08)	8050±375.27 (09)	10425 ±1326.88 (08)	1.663 (NS)
	BL	6700±467.26[a] (07)	2850±370.80[b] (06)	5325±525.0[a] (07)	18.064 (P <0.05)
Desmids	YL	NIL	NIL	NIL	NIL
	BL	7425±306.52[c] (09)	4650±233.63[b] (09)	3600±285.77[a] (08)	50.897 (P <0.05)
Rotifers	YL	495±56.93[a] (12)	561±70.28[a] (12)	947±145.98[b] (10)	6.087 (P < 0.05)
	BL	172±24.98[a] (18)	228±32.01[a] (17)	354±28.69[b] (20)	10.315 (P <0.05)
Copepods	YL	324±67.23[a] (03)	810±130.36[b] (04)	270±13.39[a] (04)	12.232 (P <0.05)
	BL	236±57.89 (07)	331±30.88 (06)	198±38.75 (05)	2.499 (NS)
Cladocera	YL	172±32.12[a] (04)	168±16.46[a] (03)	116±12.86[a] (04)	6.092 (P < 0.05)
	BL	125±20.69[a] (05)	164±23.20[a] (07)	224±18.38[b] (07)	9.326 (P <0.05)
Ostracods	YL	38±6.42 (04)	46±8.02 (04)	28±2.06 (03)	1.891 (NS)
	BL	10±3.5[b] (04)	46±4.08[a] (06)	58±8.75[a] (04)	19.358 (P <0.05)

Plankton	Seasons		
	Monsoon	Winter	Summer
Cyanophyceae	P < 0.05	P < 0.05	P < 0.05
Chlorophyceae	NS	NS	NS
Bacillariophyceae	NS	NS	NS
Euglenophyceae	NS	P < 0.05	NS
Desmids	Nil	Nil	Nil
Rotifers	P < 0.05	P < 0.05	P < 0.05
Copepods	NS	P < 0.05	P < 0.05
Cladocera	NS	P < 0.05	NS
Ostracods	P < 0.05	NS	NS

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Cladoceran density did not show significant seasonal variation in YL in both the years whereas they showed significant variation in their density in BL in both years. There was no significant difference between cladodoceran population of YL and BL in all the seasons of the study period.

The seasonal variation in density of ostracods in 2002 was significant in both lakes whereas in 2003, it was significant only in BL but not in YL.

Discussion

In the present study seasonal variation in different physico-chemical parameters, which are known to influence well being of fish and growth of plankton have been studied, to find out whether differences in these parameters in two lakes is accompanied by difference in growth co- efficient and relative condition factor of a major carp *C. catla.*

In the present study water temperature of YL ranged from 25.03°C to 26.64°C in both the years where as that of BL was 25.34°C to 27.37°C. Since a range of 28-32°C in tropical waters (IFAS: Insttitute of food and agricultural sciences, University of Florida,Circular-1051, Jinghran ,1968) is congenial for optimal growth of fish, both the lakes under study showed temperature closer to the lower limits of the optimum range. Similarly, the DO content of both the lakes during entire study period was conducive for fish growth as it was well above the minimum required amount (i.e. 5mg/lit., Alabaster & Lloid, 1980). Since higher levels of total suspended solids clog the fish gills, their concentration less than 25mg/lit is preferred (Maitland, 1990). In our study TSS level was well within the range in both lakes. However, other physico-chemical parameters showed significant difference between two lakes and some of them were in undesirable level in YL compared to BL. For instance, low turbidity (20-30 NTU) is desirable for fish culture (Zweig, 1989) as high level turbidity affects the photosynthetic process and there by the potential yield of the lake (Sukumaran & Das 2005). In BL turbidity level was within the desirable range (9.3 NTU – 33.04 NTU) whereas that in YL was (50.77NTU – 76.33NTU) higher than desired range. Similarly higher alkalinity (pH >9) in water bodies is unsuitable for good fish production (Boyd 1979). The water pH in YL was always higher than 9 whereas in BL it was less than 9 in majority of the seasons. Likewise total alkalinity in YL (929.34 mg/lit to 1016.5 mg/lit) was remarkably higher than optimal range (100 to 400 mg/lit, Schroeder, 1980) for fish culture. Whereas in BL, excepting monsoon it was within desired range of alkalinity preferred for fish culture.

BOD indicates the presence of organic load in a water body and waters having BOD more than 35 to 45 mg/lit are not good for fish culture (Pande & Sharma 1999). In the present study BOD level in YL exceeded the preferred range in all the seasons except in winter in contrast to BL wherein it was in preferred range in all seasons except monsoon.

Phosphate is a nutrient which causes rich phytoplankton crop (Moss, 1993). An optimum level 0.1 to 0.2 mg/lit phosphate (Sreenivasan, 1965) is needed for growth of plankton. In our study, in YL the phosphate concentration was several folds higher than optimal level (0.1 to 0.2 mg/lit, Sreenivasan, 1965) needed to support phytoplankton growth. Whereas in BL it was within desirable range except in monsoon.

Nitrite could be hazardous to fish if it exceeds the permissible range (Train & Russel 1979) which is 0.015 mg/lit for salmonids (Iwama et al. 2000) and generally 0.1mg/lit considered tolerable range in tropics (Hart & Reynolds, 2002). In the present study YL

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exceeded the tolerance limit whereas in BL it was very well with in the desirable range throughout the year. Minimum level of nitrate required for the lake to be productive is 0.1mg/lit (Srinivasan 1965, Hart & Reynolds, 2002). In the present study nitrate content although exceeded the optimal level in both the lakes, the concentration of nitrate was far higher in YL than BL. The excessive level of nutrients in YL was reflected in the presence of algal bloom during most part of the study period.

Unionized ammonia in the range of 0.02 – 0.2mg/lit is toxic (Alabaster & Lloyd, 1980., Joseph et al. *1993*) to fishes as excessive ammonia in water tends to block O_2 transfer from gills to the blood (Smart, 1978). In the present study ammonia content in YL was not only significantly higher than BL but was also in toxic level.

Hydrogen sulphide in water bodies is another indication of pollution (Oslen & Sommerfeld 1977). In the present study H_2S is the only parameter, which was in undesirable range in both the lakes. It ranged from 2.58 mg/lit to 5.29 mg/lit in YL and from 0.56 mg/lit to 1.01 mg/lit in BL. High levels of pH, total alkalinity, turbidity, BOD, H2S, phosphate and nitrite were reported in number of studies in different lakes in India and outside, to cite a few, Hutchinson 1957, Verma 1967, Banergia 1967, Saxena & Adoni 1973, Ayyappan & Gupta 1981, Yousuf et al. 1986, Kaur et al. 2000, Ragavendra & Hosmani 2002. However these studies did not focus on the fact that whether these conditions interfered with growth and well being of fish in these water bodies. The isometric growth of fish under optimum conditions follows length-weight relationship, wherein weight is cube of length.(cube rule , Le Cren, 1951). In the length weight relationship equation ($W=aL^b$), b is the growth co-efficient and its value is 3 (Allen, 1938) under optimal conditions. Hile (1936) and Martin (1949) opined that value of b usually lies between 2.5 and 4. Hence, in the study of length weight relationship, value of b because less than 2.5 can be considered as subnormal growth of fish in that given lake. Further the relative condition factor (Kn) is an expression used to asses the condition of fish, and Kn value 1 or more than 1 is considered as well being of fish. The present study, which compares these parameters of *C. catla* in two lakes, for two calendar years, reveals a few interesting facts. The growth coefficient (b) was 3 or close to 3 in majority of seasons, accompanied by Kn value 1 are more than 1 in BL, where as it was less than 3 accompanied by Kn value less than 1 in all the seasons in YL. These observations clearly indicate better growth and health (well being) of *C. catla* in BL than in YL. Although several earlier studies on fish growth reveled sub optimal growth of fish they did not provide evidence of any causative factor. In the present study, the sub optimal growth of *C. catla* in YL was accompanied by high pH, turbidity, total alkalinity, BOD, nitrate, nitrite, phosphate, hydrogen sulphide and ammonia which were beyond normal range for fish culture in contrast to lower values of these parameters accompanied by normal growth of *C. catla* in BL. Higher levels of these physico-chemical factors directly or indirectly interfere with fish physiology and affect their growth. For instance high turbidity (Zweig, 1989) reduces photosynthetic zone resulting in night time decline of DO and higher pH (Boyd, 1979) influences the blood pH and causes alkalosis; damages skin, gills and eyes; and increases mucus production. Similarly, oxygen consumption of fish is affected by high nitrite, nitrate and ammonia (Tilak et al. 2005) as nitrate in addition high ammonia interferes with oxygen transport from gills to blood (Smart, 1978.,Lewis & Morris, 1986., Datta et al. 2005) and damages gills. Sub-optimal levels of unionized ammonia (0.1 – 0.42 mg/lit) causes significant variation in condition factor (Datta et al. 2005). Likewise higher than tolerable level of hydrogendulphide might cause death to the fishes or at the very least

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stress (Barthelmes & Bramick 2003). The combined effect of all these physico-chemical factors might induce stress response as suggested by Iwama (2000). It is well known that stress adversely affects growth of animals. Hence, the sub optimal growth of *C. catla* as indicated by deviation from cube rule and lower Kn values in YL are due to prevailing physico-chemical conditions in YL. This view is further supported by the fact that the above parameters which are in normal range in BL in which not only growth co-efficient of *C.catla* obeyed the cube rule but also Kn values indicated well being of fish.

The difference in physico-chemical characteristic in two study lakes was also reflected in plankton density and diversity; indicating water contamination in YL. Although phytoplankton being producers play a key role in aquatic food chain, higher nutrient levels cause their bloom, which will be detrimental to fish by various effects. Nutrient enrichment resulting in algal bloom is indicated by excessive growth of certain algal s genera ; *Microcystis , Anabaena, Oscillatoria, Scenedesmus, Pediastrum, Fragellaria etc*. (Palmer, 1980., Bush & Welch, 1972). In the present study YL showed algal bloom throughout the year and was dominated by cyanophytes, especially *Microcysties , Oscilotoria, Anabina* etc and bacillariophytes such as *Navicula, Nitzschia, Synedra* etc, which indicate nutrient load and sewage pollution (Palmer,1980). This view is also supported by the presence high density of rotifers viz. *Brachionus angularis, B. quadricormis, Keratella cochlearis, Felinia longiseta, Polyarthra vulgaris* and *Conochilus dassaurius*, which are also an indicator of high nutrient load (Sharma et al. 1999., Bahura et al. 1993) . In contrast in BL, low nutrient levels compared to YL was accompanied by the presence of desmids, high density of chlorophyceae which grow better in waters with low organic matter and high DO (Goldman & Home, 1983). Cyanophytes dominated by *M. aeruginosa* are found to produce two toxins viz; hepatotoxin , microcystin and a neurotoxin, anatoxin and adversely effect the well being of the fish (Ballot et al. 2003). Cyanophycean bloom also causes “off flavor” either by producing a substance called MIB (Methyl isoborneal) or by the decomposition process of their own counterparts (Martin, et al; 1994). In our study we observed bloom and off flavor in YL quite often . In addition, plankton diversity was more in BL than in YL. Put together these biological parameters indicate better conditions in BL than in YL. Hence the study by comparing growth

co-efficient and relative condition factor of same species in two water bodies which difffer in physico-chemical properties, first time provides an evidence for the fact that water quality parameters in undesirable range in natural water bodies interfere with growth and well being of fish . It is to be noted that though the conditions in YL were never severe enough to cause fish deaths, they interfered with growth and well being of *C.catla.* Hence such studies will be useful in assessing the suitability of ponds for fish culture.

Acknowledgements. First author is grateful to University Grants Commission, India, for the award of teacher fellowship under the faculty improvement program.

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BIO-REMEDIATION OF REFINERY EFFLUENTS BY STRAINS OF PSEUDOMONAS AERUGENOSA AND PENICILLIUM JANTHINELLUM

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(Received 27th February 2007; accepted 14th February 2008)

Abstract. A study was conducted to evaluate the potentials of *Pseudomonas aeruginosa* and *Penicillium janthinellum* and their mutants in degradation of crude oil in river Kaduna effluents after two weeks incubation at 30°C, Degradation potentials ranged from partial to good. A mixture of the pure strains and mutants (subjected to 10-minute irradiation) of the two micro-organisms, as well as the pure strains of *Pseudomonas aeruginosa* showed the best degradation. Five and fifteen minute mutants of the two micro-organisms, as well as the pure strains of *Penicillium janthinellum* showed partial degradation. Against pristine and phytane as biomarkers, Carbon 23 (C_{23}) did not appear in the chromatogram of effluents that had undergone partial or good degradation.All consortia were observed to have significant decreases in contents of phenol, oil and grease, phosphates, ammonia, nitrates, and sulphates after two weeks of incubation at 30°C. A comparative analysis of the effluent after two weeks of incubation in relation to FEPA specifications and KRPC treated waste water (TWW) after bioremediation, revealed that, in River Kaduna water sample the phosphate concentration of most consortia were greater than TWW, and FEPA limits, except for consortia G^1 and H^1 that were lower. Other physicochemical parameters showed a lower concentration compared to that of TWW. At the end of experiment, all the consortia except G^1 and H^1 were lower than the FEPA limits for oil and grease. Similar occurrence was observed in phenol concentration for all the consortia.
Keywords: *Biodegradation, hydrocarbons, micro-organisms, physico-chemical characteristics*

Introduction

Nigeria became an exporter of oil when production reached 6000 barrels per day. The current daily production is over two million barrels [5, 13]. Crude oil exploration and production, petroleum refining and marketing operations have several attendant environmental problems [2]. Production effluents consist mainly of produce formation waters emanating continuously from different oil bearing formations together with crude oil and associated gas or condensate [17]. Produce formation waste poses great danger when disposed into fresh waters because of its salinity, heavy metal and Polycyclic Aromatic Hydrocarbons (PAHs) content [21, 20]. Wastewater released by crude oil processing and petrochemical industries are characterized by the presence of large quantities of crude oil products, polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface-active substances, sulfides, naphthylenic acids and other chemicals [19, 21]. Due to the ineffectiveness of purification systems, wastewaters may become seriously dangerous, leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem [7].

Various studies have shown positive correlations between pollution from refinery effluents and the health of aquatic organisms. Previous observations [12], suggested a correlation between contamination of water and sediments with aromatic hydrocarbons from refinery effluents, and compromised fish health. An earlier study [16] demonstrated the accumulation of heavy metals with accompanying histopathology in

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Oreochromis niloticus exposed to treated petroleum refinery effluent from the Kaduna Refinery and Petrochemical Co. Ltd.

Wastewater containing high concentrations of pollutants or having uncomfortable pH is always more difficult and highly expensive to treat [14]. This indicates the need for a more efficient and cheaper secondary method of cleaning up wastewater.

Bioremediation is a new method of oil spill clean up that is far more effective than any of the mechanical methods used [1, 6, 20]. This involves the use of micro-organisms ('Petrophiles') to breakdown complex materials into simple end products. These products exist either naturally in the environment or are artificial ("xenobiotic") products [9]. Biodegradability is important for determining the behaviour of such chemicals in the environment. Within the ecosystem, micro-organisms have evolved a host of enzymes that aid in biodegradation of natural products.

This microbial clean-up method cleans the oil as well as a number of other harmful pollutants and is perhaps the best, most environmentally safe process used today. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a food source [9]. This makes them singularly qualified for cleaning oil spills and even tanker bottoms containing oil residue. In bioremediation, several different types of these micro-organisms are used. Bioremediation efforts have been hardly, if at all applied in the Nigerian, and possibly West African situation. In view of the fact that Nigeria is now a major player in the oil industry, and with regards to environmental concerns that are increasingly being expressed, it has become more and more important to find solutions to problems of poor environmental management. The objective of this study therefore is to evaluate the efficiency of pure and mutant strains of two micro-organisms (*Pseudomonas aerugenosa* and *Penicillium janthinellum*) in degradation of hydrocarbons and other potentially harmful substances from the Kaduna Refinery and Petrochemical Company

Materials and methods

Study site

The Kaduna Refinery and Petrochemical Company (KRPC) occupies a land area of 2.89 square kilometres approximately 15km Southeast of Kaduna city [11] (See map; Fig 1). Its location has an elevation of approximately 615m above mean sea level [8]. Kaduna Refinery was constructed by the Chiyoda Chemical Engineering and Construction Company (now Chiyoda Corporation) and was commissioned in 1980 with an initial capacity of 100,000 BPSD as the third Refinery in Nigeria in order to cope with the tremendous and growing demand for petroleum products [11]. In December 1986, the design capacity of the fuels plants of the Refinery was successfully increased by an additional 60,000 BPSD to the initial 50,000 BPSD, bringing the total refinery installed capacity to 110,000 BPSD [11].

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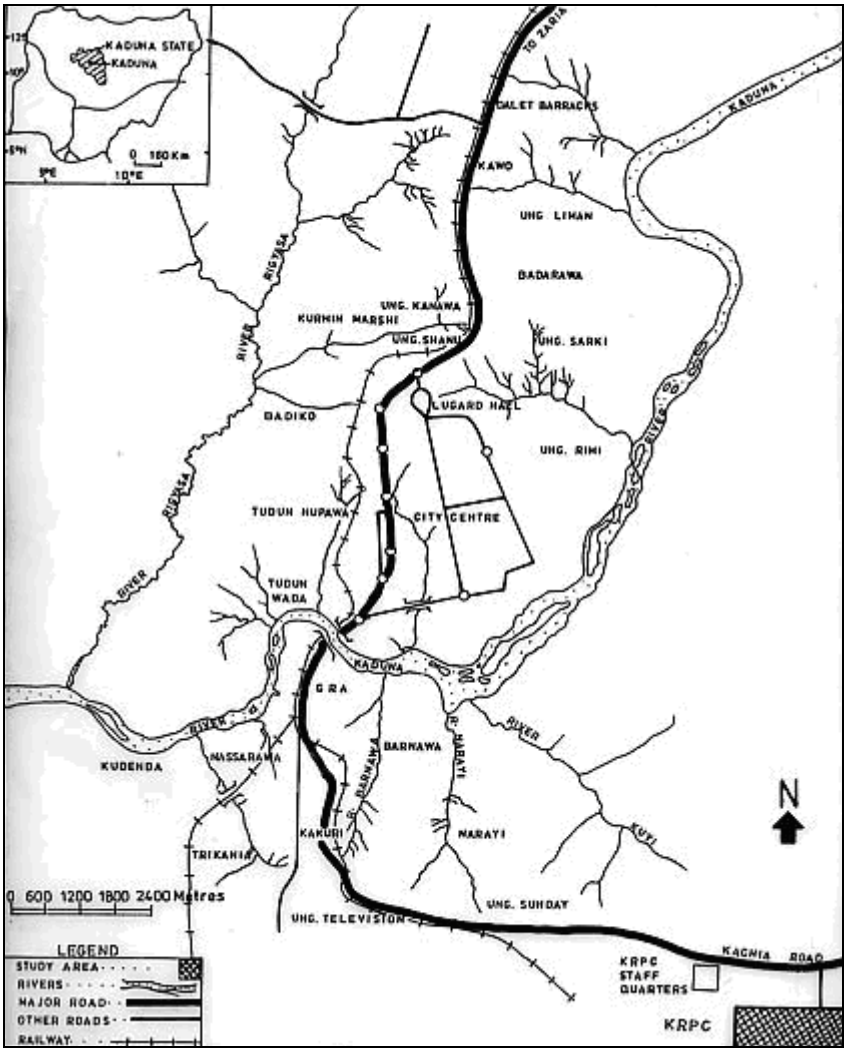

Figure 1. *Map of Kaduna city*

The Refinery was designed to process two types of crude oils: the imported heavy crude and Nigerian light crude into fuels and lubes products [11]. The Nigerian light crude which is basically naphthenic is reserved for the manufacture of fuel products and the imported heavy crude, that is paraffinic is on the other hand, used mainly for the production of lubricating oils, waxes and asphalts [11]. Consequently, the refinery has two process sections: the Fuel section and the Lubricating Oil, wax and Asphalt section [8].

Microorganisms

Pseudomonas aerugenosa and *Penicillium janthinellum* were obtained from the stock culture of the Department of Pharmaceutical Sciences and Department of Microbiology respectively.

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Induction of mutant strains

A Phillip's germicidal Lamp (of 254nm short wavelength) was used to induce mutations in three out of 4 batches each of *Pseudomonas aerugenosa* and *Penicillium janthinellum* grown in Petri dishes. Three batches from each species were irradiated for 5, 10, and 15 minutes. Mutants were immediately transferred into fresh Petri dishes and allowed to grow for 8 days before they were used in the experiment.

Crude oil, refinery effluent and River Kaduna samples

Samples of effluent and river Kaduna water were respectively collected separately using 2L, 4L and 50L sterile polyethylene vessels at KRPC refinery, Kaduna and River Kaduna, while crude oil was collected from fuels laboratory, KRPC. Samples of KRPC effluents and River Kaduna water were transported in ice chests and analysed for some physicochemical parameters when the temperature of samples had normalized within 2 weeks of cultivation.

Inocula development

Colonies of Pseudomonas aerugenosa (Ps) and Penicilium janthinellum (Pe) and their mutants (Ps5, Ps10, Ps15, Pe5, Pe10, Pe15) in different combinations of Ps alone, Pe alone, PsPe, Ps5Pe5, Ps10Pe10, and Ps15Pe15 were transferred respectively from agar plates to 6 containers containing 1.5g of fertilizer, 100ml of wastewater and 400ml of River Kaduna samples. These media were manually shaken and cultivated for 24 hours at room temperature. Of these, grown cultures were used to inoculate fresh culture media, which were cultivated at the same conditions for 2 weeks.

Culture medium

One hundred milligrams (100ml) of waste water samples was supplemented with 1.5g of fertilizer containing 660ppm of NH_4NO_3:460 ppm of PO_4^{-2} (1:4:1) in 1L of water from river Kaduna water.

The control experiment

Control I: 100ml of Treated Waste Water samples + 1.5g of fertilizer + 1L of Distilled water. Control I: 100ml of Treated Waste Water samples + 1L of water from river Kaduna. Control III: 100ml of Treated Waste Water samples + 1.5g of fertilizer + 1L of water from river Kaduna.

Experimental set up

Thirty six (36) polyethylene containers (3L each) with caps were used throughout the course of this experiment. Nine (9) of these containers were used for inocula development, 18 containers for the culture medium (i.e. 6 containers with 3 replicates each), while the remaining 9 served as the control (i.e. 3 containers with 3 replicates each). These set ups were arranged in a Randomized Complete Block Design at room temperature of 25 – 30°C in the Laboratory.

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Gas/liquid chromatographic determination of oil biodegradation

Nine set ups each of three replicates for the investigations were: (1). Pseudomonas aerugenosa, (2) Penicillium janthinelum, (3): Pseudomonas aerugenosa and Penicillium janthinelum,(4) Pseudomonas aerugenosa and Penicillium janthinelium Irradiated with 254nm UV lamp for 5 minutes, (5) for 10 minutes, (6) for 15 minutes, (7) Control I, (8) Control II, (9) Control III. Each set up was replicated three times.

Ten milliliters (10ml) of water from river Kaduna was added to each test tube. Next, 1ml of crude oil was added to each test tube and allowed to form a thin layer over the water surface. Control experiments were (7) Control I: 5ml of Distilled water + 1ml of Crude Oil; (8) Control II: 10ml of River Kaduna + 1ml of Crude Oil; (9) Control III: 10ml of River Kaduna + 0.5g of Fertilizer. With a sterile pipette, Set-up No. 1 was inoculated with 2ml of the Pseudomonas aerugenosa, Set-up No. 2 was inoculated with 2ml of Penicilium janthinellum, Set-up No. 3 was inoculated with 1ml of both microbes, and Set-ups Nos. 4, 5, and 6 were inoculated with 1ml of UV irradiated strains both microbes. A cap was placed on each tube and each was inverted several times to allow the micro-organisms to mix with the crude oil. Caps were loosened one-half turn and the set-ups were incubated at 30^{o}C. Each tube was observed and inverted every 24 hours for 16 days. Each set up was subjected to a Gas/Liquid Chromatographic analysis.

Results

Table 1 presents a summary of the results for the degradation of crude in river Kaduna effluents after two weeks incubation at 30°C, by *Pseudomonas aeruginosa* and *Penicillium janthinellum* and their mutants in different consortia. Degradation potentials ranged from partial to good. A mixture of the pure strains and mutants (subjected to 10-minute irradiation) of the two micro-organisms, as well as the pure strains of *Pseudomonas aeruginosa* showed the best degradation. Five and fifteen minute mutants of the two micro-organisms, as well as the pure strains of *Penicillium janthinellum* showed partial degradation.

Figures 2-5 show typical chromatographic profiles of the effluents under no inoculation, no degradation, partial, and good degradation conditions after two of weeks incubation at 30°C respectively. Against pristine and phytane as biomarkers, Carbon 23 (C_{23}) did not appear in the chromatogram of effluents that had undergone partial or good degradation.

All consortia were observed to have significant decreases in contents of phenol, oil and grease, phosphates, ammonia, nitrates, and sulphates after two weeks of incubation at 30°C (Tables 2-7).

A comparative analysis of the effluents after two weeks of incubation (Table 8) in relation to FEPA specifications and KRPC treated waste water (TWW), revealed that, the phosphate concentration of most consortia were greater than TWW, River Kaduna and FEPA limits, except for consortia G^1 and H^1 that were lower. Other parameters showed a lower concentration compared to that of TWW. At the end of experiment, all the consortia except G^1 and H^1 were lower than the FEPA limits for oil and grease. Similar occurrence was observed in phenol concentration for all the consortia.

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Table 1. *Gas/liquid chromatographic analysis of crude oil in a bioremediation scheme using Pseudomonas aerugenosa and Penicillium janthinellum and their mutants*

Consortia	Degradation rating
A^1 (E + Rk + N + *Pseudomonas aerugenosa*)	D
B^1 (E + Rk + N + *Penicillium janthinellum*)	C
C^1 (E + Rk + N + PePs)	D
D^1 (E + Rk + N + Pe_5Ps_5)	C
E^1 (E + Rk + N + $Pe_{10}Ps_{10}$)	D
F^1 (E + Rk + N + $Pe_{15}Ps_{15}$)	C
G^1 (E + Dt)	B
H^1 (E + Rk)	B
I^1 (E + Rk + N)	C

Note: B: No Degradation, C: Partial Degradation, D: Good Degradation. E: Effluent, Rk: Water from river Kaduna, N: N.P.K fertilizer, PePs ($_{5,10,15}$): *P. aerugenosa* and *P. janthinellum* irradiated at 5, 10 and 15 minutes of UV (254nm).

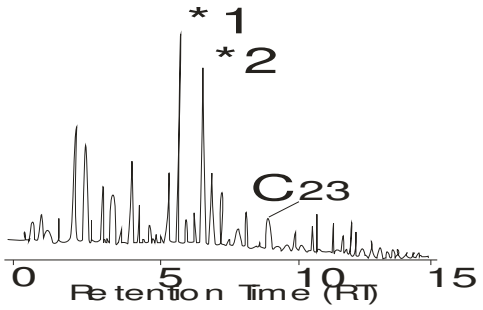

Figure 2. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates without inoculation (Sterile, A) with Pseudomonas aerugenosa and Penicillium janthinellum after two weeks at 30°C.*

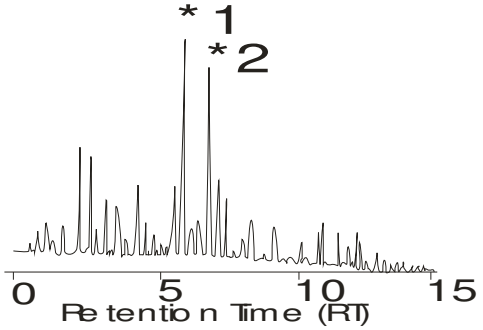

Figure 3. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates showing no degradation (B) after two weeks incubation with Pseudomonas aerugenosa and Penicillium janthinellum at 30°C.*

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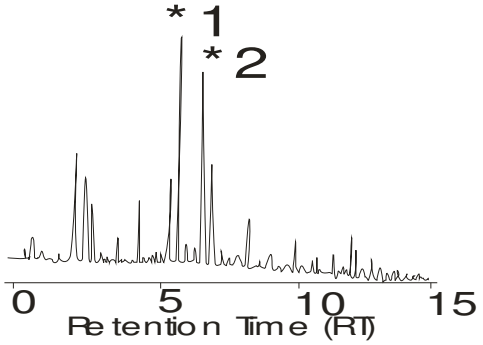

Figure 4. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates partial degradation (C) after two weeks incubation with Pseudomonas aerugenosa and Penicillium janthinellum at 30°C.*

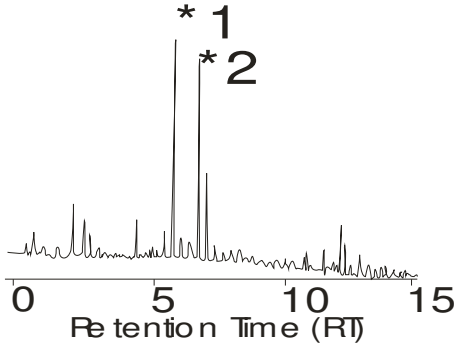

Figure 5. *Gas/liquid chromatographic profile of KRPC petroleum effluent for isolates showing good degradation (D) after two weeks incubation with Pseudomonas aerugenosa and Penicillium janthinellum at 30°C.*

Table 2. *Oil and grease (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	**A^1**	**B^1**	**C^1**	**D^1**	**E^1**	**F^1**	**G^1**	**H^1**	**I^1**
0	12.48^c	10.80^c	11.40^c	12.50^c	12.60^c	12.00^c	12.30^c	11.40^c	11.80^b
4	10.23^b	8.47^b	7.49^b	10.48^b	8.59^b	10.75^c	12.20^c	11.20^c	9.30^b
8	9.74^b	6.37^a	6.91^b	10.15^b	6.45^a	10.25^b	12.01^c	11.01^c	7.01^b
12	9.48^b	4.75^a	5.30^a	9.13^b	4.41^a	9.67^b	11.50^c	10.60^c	5.52^a
16	9.25^b	3.45^a	4.80^a	7.87^b	3.74^a	8.95^b	11.01^c	10.20^b	3.61^a

Values with similar superscripts do not have any significant differences within columns.**Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.

[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

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Table 3. *Phenol concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	3.21^c	1.94^b	3.04^c	2.78^c	3.10^c	2.48^b	1.48^b	2.14^b	1.79^b
4	0.90^a	0.00^a	0.71^a	0.87^a	0.30^a	2.01^b	1.24^a	1.94^b	0.68^a
8	0.67^a	0.00^a	0.50^a	0.51^a	0.20^a	1.49^b	1.41^b	1.97^b	0.71^a
12	0.49^a	0.00^a	0.30^a	0.41^a	0.16^a	1.18^a	0.80^a	1.60^b	0.50^a
16	0.30^a	0.00^a	0.10^a	0.30^a	0.10^a	0.80^a	0.50^a	1.08^a	0.20^a

Values with similar superscripts do not have any significant differences within columns **Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N. [River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

Table 4. *Ammonia concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	1.48^c	1.24^c	1.35^c	0.89^b	1.41^b	0.59^b	0.57^b	1.08^c	1.44^c
4	1.34^c	1.18^c	1.21^c	0.82^b	1.02^b	0.54^b	0.56^b	1.04^b	1.21^c
8	1.14^c	0.84^b	0.75^b	0.67^b	0.52^a	0.50^a	0.50^a	0.87^b	0.87^b
12	1.04^b	0.52^a	0.65^b	0.57^b	0.48^a	0.44^a	0.50^a	0.84^b	0.51^a
16	1.02^b	0.20^a	0.50^a	0.50^a	0.45^a	0.31^a	0.50^a	0.92^b	0.20^a

Values with similar superscripts do not have any significant differences within columns.
Key: A^1: Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.
[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

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Table 5. *Nitrate concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	1.00^c	0.84^c	0.53^b	0.88^c	1.01^c	0.94^c	0.49^b	0.61^b	0.98^c
4	0.80^b	0.61^b	0.48^b	0.80^b	0.84^c	0.91^c	0.48^b	0.60^b	0.67^b
8	0.67^b	0.42^b	0.39^b	0.72^b	0.61^b	0.74^b	0.46^b	0.58^b	0.61^b
12	0.54^b	0.24^a	0.26^a	0.68^b	0.50^b	0.52^b	0.46^b	0.52^b	0.55^b
16	0.50^a	0.10^a	0.20^a	0.64^b	0.50^b	0.54^b	0.42^b	0.46^b	0.48^b

Values with similar superscripts do not have any significant differences within columns. **Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

Table 6. *Sulphate concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	20.80^d	18.70^c	19.48^d	21.04^d	20.70^d	24.00^e	5.05^a	6.19^a	21.00^d
4	20.10^d	16.60^c	15.92^b	20.14^d	18.50^c	23.03^e	5.02^a	6.10^a	18.60^c
8	19.50^d	15.20^b	15.10^b	19.70^d	16.60^c	21.13^d	5.00^a	5.98^a	17.22^c
12	18.30^c	13.70^b	14.50^b	18.90^c	15.50^b	21.00^d	5.00^a	5.96^a	16.63^c
	17.40^c	12.60^b	13.70^b	18.01^c	14.40^b	20.60^d	4.90^a	5.94^a	15.20^b

Values with similar superscripts do not have any significant differences within columns.**Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

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Table 7. *Phosphate concentration (mg/L) of KRPC effluent in a bioremediation scheme using Pseudomonas aerugenosa, Penicillium janthinellum and their mutants in water from River Kaduna*

Days	A^1	B^1	C^1	D^1	E^1	F^1	G^1	H^1	I^1
0	50.00^d	50.40^d	50.00^d	50.40^d	50.00^d	50.77^d	0.10^a	0.50^a	49.70^e
4	47.20^d	44.30^d	46.10^d	49.20^d	47.00^d	48.90^d	0.10^a	0.40^a	46.70^e
8	40.30^c	36.50^c	39.70^c	41.30^c	38.70^c	41.80^d	0.10^a	0.40^a	39.00^d
12	33.10^c	29.70^c	29.90^c	31.80^c	30.40^c	38.40^c	0.10^a	0.30^a	31.50^d
16	26.70^b	21.20^b	23.50^b	29.40^c	22.70^b	35.50^c	0.10^a	0.30^a	27.90^c

Values with similar superscripts do not have any significant differences within columns. **Key: A^1:** Rk+Ef+N+Pe**, B^1:** Rk+Ef+N+Ps, **C^1:** Rk+Ef+N+PePs, **D^1:** Rk+Ef+N+Pe_5Ps_5, **E^1:** Rk+Ef+N+$Pe_{10}Ps_{10}$, **F^1:** Rk+Ef+N+$Pe_{15}Ps_{15}$**. G^1:** Rk+Ef, H^1: Dt+Ef, **I^1:** Rk+Ef+N.
[River Kaduna water sample (Rk), Effluent (Ef), Nutrient (N), *P. janthinellum* (Pe), *P. aerugenosa* (Ps), Mutants ($Pe_{5,10,15}$ $Ps_{5,10,15}$), Distilled water (Dt)]

Table 8. *Comparative physico-chemical quality [mg/L]of KRPC treated wastewater (TWW), River Kaduna water and consortia after bio-remediation*

	Oil and grease	Phenol	Ammonia	Nitrate	Sulphate	Phosphate
TWW	13,50	3,45	1,48	0,50	18,80	5,80
River Kaduna water	0,05	<0.1	0,78	0,05	2,02	0,01
FEPA Limitation Guideline (1991)	10,00	0,50	0,20	0,20	NI	NI
A^1	9,25	0,30	1,02	0,50	17,40	26,70
B^1	3,45	0,00	0,20	0,10	12,60	21,20
C^1	4,80	0,10	0,50	0,20	13,70	23,50
D^1	7,87	0,30	0,50	0,64	18,01	29,40
E^1	3,74	0,10	0,45	0,50	14,40	22,70
F^1	8,95	0,80	0,31	0,54	20,60	35,50
G^1	11,01	0,50	0,50	0,42	4,90	0,10
H^1	10,20	1,08	0,92	0,46	5,94	0,30
I^1	3,61	0,20	0,20	0,48	15,20	27,90

Discussion

The synergy of microbial systems was effective in the degradation of pristane and phytane (biomarkers) as well as other hydrocarbons in the consortia.The highest percentage (70%) break down of oil and grease was observed in C^1 . This explains the synergistic potentials of the combined culture of the bacterium (*P. aerugenosa*) and fungus (*P. janthinellum*) in this bioremediation. Consortia I^1 was also very effective in oil and grease biodegradation. FEPA [10] requirements for oil and grease in wastewater release/discharge were met by most microbial systems except for the controls, which had very low biological activities.

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Significant decreases in phenol concentration of all consortia (especially the controls G^1 and H^1) agree with a report (15) that there was decrease in phenol in consortia with low biological activities because of the volatility of phenol (21). Weathering/evaporation may have contributed to the loss of phenol in these consortia (15). The efficacy of consortium B^1 that contained effluent, River Kaduna water sample, NPK fertilizer and *Pseudomonas aerugenosa* in this bioremediation scheme, was confirmed by the 100% degradation of phenol. This bacterium has often been cited as one of the most efficient phenolytic micro-organisms [16] due to its capability to transport and metabolize phenols and other hydrocarbons [7]. All consortia except consortia F^1, G^1 and H^1 met the FEPA requirements/guideline for treated wastewater.

The effectiveness of bio-augmentation, bio-stimulation and/or synergistic potentials of combined microbial cultures in bioremediation was observable in the cases of Nitrates, Ammonia, and Sulphates. In the case of phosphates only the colonies irradiated at 254 nm for 10 minutes showed significant biodegradation. Mutants responsible for the biodegradation of sulphates and phosphates may be responsible for the breakdown in these physicochemical parameters. Phosphate is a growth-limiting factor of micro-organisms (3). This could be the reason for the early removal of phosphates in consortia like D^1 and I^1 with low biological activities at the commencement of the experiment. However, very high phosphate concentration was observed as compared to effluents without fertilizer. This could therefore be due to the addition of fertilizer.

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MICROBIAL POPULATIONS DURING COMPOSTING PROCESS OF ORGANIC FRACTION OF MUNICIPAL SOLID WASTE

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(Received 1st February 2007; accepted 24th March 2008)

Abstract. Composting is one of the more economical and environmentally safe methods of recycling waste generated by the consumer society. Due to the complexity of substrates and intermediate products, microbial diversity and the succession of populations is a prerequisite to ensure complete biodegradation. In the present work, we studied the succession of microbial populations during composting process of organic fraction of Municipal Solid Waste and some physical and chemical parameters were followed during process, moisture content was maintained at 50-60% and temperature monitored daily, in order to study the effects of important environmental factors on microbial communities. The results showed that the substrate was colonized in major proportion by bacteria (44.6%), actinomycetes (32.3 %) and in lower number by fungi (23.1%), mainly represent by the following dominant genera: *Bacillus, Streptomyces, Actinomyces*, *Pseudomonas and Azospirillum.* The Multiple Regression Analysis, reveal that the environmental parameter that major influence made on the microbial groups mentioned was the temperature.
Keywords: *compost, microbial communities, bacteria, actinomycetes, fungi*

Introduction

Composting is an aerobic process by which organic materials are degraded through the activities of successive groups of microorganisms; it is an environmentally sound way to reduce organic wastes and produce organic fertiliser or soil conditioner (Gajdos, 1992). Composting of the organic fractions of municipal solid waste (MSW) is therefore expected to increase substantially in many conuntries, parly due to legislative changes (Barth and Kroeger, 1998).

Although composting is a microbiological process, little is known about microorganisms involved and their activities during specific phases of the composting process. Definig the diversity and structure of microbial communities of compost through their constituent populations has been of considerable interest to compost researchers in order to address basic ecological questions such as how similar are microbial communities in mature compost that were made from different feedstocks and using different composting methods (Tiquia and Michel, 2002). Different microbial communities predominate during the various composting phases (mesophilic and thermophilic), each of which being adapted to a particular environment. The

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composition of the microbial communities during composting is determined by many factors (temperature, pH, water content, C/N, etc). In adition under aerobic conditions, temperature is the major selective factor for populations and determines the rate of metabolic activities.

Despite the use of growth requiring culturing techniques is often disputed, while the culture-independent molecular screening techniques will definitely detect numerous unique microorganisms, a few references clearly indicate that culturing studies still deliver non-overlaping information, consequently none of the two approaches can claim to be superior to the other yet (Brambilla *et al.,* 2001; Gurther *et al.,* 2001; Dees and Ghiorse, 2001).

The aim of this study was reveal the succession of microbial populations during a whole composting process and the influence of some physical and chemical parameters under microbial concentrations. The composition of microbial communities was investigated by conventional cultured techniques.

Materials and methods

Compost site and sampling

Organic fraction of Municipal Solid Waste (MSW) compost was sampled in March 2005 at a small scale composting plant in Landfill "Calle 100" (Havana City). The waste was processed in open windrows of about 15 m length, 2m of width and a height of 1m. The windrows were turned two times per month with a compost turner. Samples were taken daily the first four days and weekly during rest of the time (26 weeks) at a deep of 15cm below the surface, according to Olynciw (2002). From each pile three samples were taken from separated positions (about 5m distance), each of these samples was composed of three subsamples that were bulked after sieving (<8mm mesh). Samples were transporter to the laboratory rapidly and all the trials were made in the same day.

Physical and chemical parameters measured

Temperature of the piles was monitored daily during the process using composting thermometers inserted at different heights in the piles. The water content was determined by drying of samples at 105ºC for 48 h. For pH and electric conductivity measurement, a solid-liquid extraction was carried out: compost samples were diluted 1:10 in distilled water, placed in a shaker for 24 h and finally a vacuum filtration was made.

Isolation of microbial cultures from compost

The compost sample (5g) was diluted in 45 mL of buffer solution (0.06M Na_2HPO_4/NaH_2PO_4) (1/9 v/v), pH 7.6. Decimal serials dilutions (10^{-1} to 10^{-10}) were made and inoculated aseptically in Petri dishes (10 µL for plate) with different culture media: Potato Dextrose Agar (PDA), Nutrient Agar (NA) and Starch Ammoniacal Agar (SAA); in order to facilitate the growth of fungi, bacteria and actinomycetes respectively. Petri dishes were incubated at 30°C(mesophilic microorganisms) and 50°C (termophilies) for 72h (PDA), 37°C or 50°C for 24h (NA) and 37°C or 55°C for 120 h (SAA), according to the phase were the isolation was carried out. After incubation isolated colonies of bacteria, fungi and actinomycetes were selected. The evaluation of

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cellular concentration in a compost samples was determined by plate counting of serials dilutions according to equation 1:

$$CFU/g = Colonies\ Numbers \bullet dilution \bullet 100 \quad (Eq.1)$$

Characterisation of isolates

Conventional morphological criteria and biochemical test were made to pure culture of bacteria and actinomycetes according to Bergey's Manual (1994). Identification of fungal species and genera were carried out according to Raper and Fenell (1965); Ellis, (1971, 1976) and Bissett (1991). All the isolated were phenotipically characterized by the API 20B test System.

Statistical analysis

The influence of physical and chemical parameters [temperature, pH, water content and electric conductivity (EC)] on the microbial concentration was tested statistically by means of the analysis of variance (ANOVA) and multiple regression analysis using the Stargraphics Plus v.3.0 program.

Results

The following groups of microorganisms were found in organic waste compost microbial complex: Gram-negative aerobes of genera *Pseudomonas*, *Azotobacter*, *Azospirillum* Gram-positive aerobes of genera *Micrococcus*, Gram-negative microorganisms included spore forming bacteria of the genera *Bacillus*, microorganisms of actinomycetes line (Gram-positive bacteria) *Streptomyces, Actinomyces* groups. Among the microscopic fungi that were also found; *Trichoderma, Alternaria, Penicillium, Ulocladium* and *Aspergillus.* Microbial populations densities were usually high (10^{8}-10^{11} CFUg $^{-1}$ of compost). *Fig. 1* shows the composition of the microbiota in MSW compost, as well the frequency of appearance of the microbial genera.

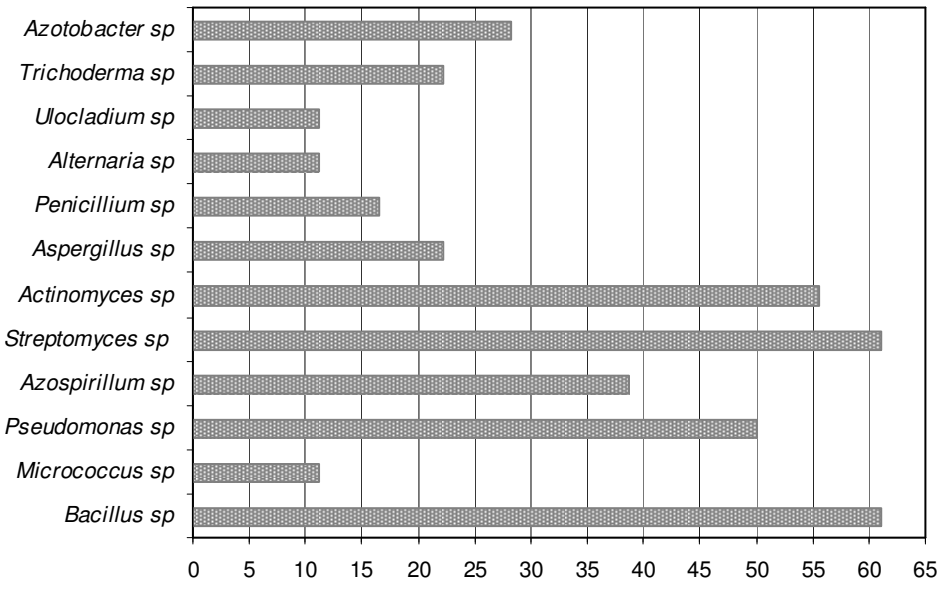

Figure 1. *Composition of the microbiota (%) in municipal solid waste compost.*

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In the analysis of the data on microbial diversity we considered as dominating those species of microorganisms whose relative abundance excceded or reached about 30% (Dobrovol´skaya *et al.,* 1997). MSW community had two genera of dominant microorganisms *Bacillus* and *Streptomyces* (61.1%), followed by *Actinomyces* (55.5%), *Pseudomonas* (50%), *Azospirillum* (38.8%). Many of those genera are also members of soil microbiota; these results are not surprising, due to the similarity between compost and a soil in good conditions as to physical-chemical characteristics. The fungal specie *Ulocladium atrum* Preuss, is consider as a new report for MSW compost.

The substrate was colonized in major proportion by bacteria that represents (44.6%), followed by actinomycetes (32.3 %) and in lower number by fungi (23.1%). Bacteria are nutritionally also the most diverse group of compost microorganisms, using a broad range of enzymes to chemically degrade a variety of organic materials, as a result, numbers of bacteria are usually much higher than number of others microorganisms, e.g. fungi (if total numbers are comparable at all). Consequently, bacteria are responsible for the most of initial decomposition and heat generation in compost, provide the major growth requirements are met. The succession of microbial populations (bacteria, fungi and actinomycetes) during compost process is shown in *Fig. 2.*

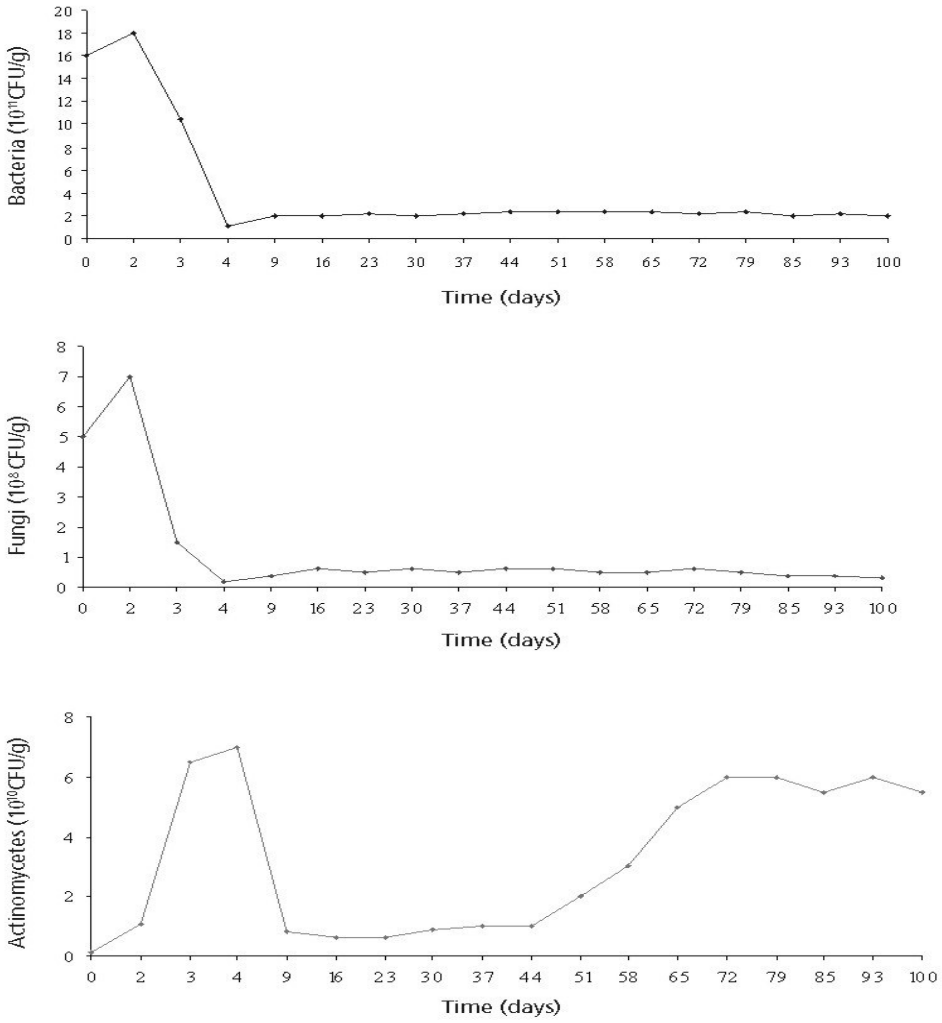

Figure 2. *Succession of microbial populations (bacteria, actinomycetes and fungi) during a whole composting process.*

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Table 1 shows the values of physical and chemical parameters measured during the whole composting process. After data processing, statistical analysis demonstrated that temperature and pH were significantly at $p<0.01$(bacteria, fungi and actinomycetes), water content and electric conductivity were significantly at $p<0.01$ only for fungal concentrations. The equations obtained were:

Bacteria = 0.497793*pH–0.0542572*Temperature * (Eq.2)

Actinomycetes = -9.78774 + 1.56072*pH – 0.0300973* Temperature * (Eq.3)

Fungi=0.593048*EC+0.0256965*H_2Ocontent -0.22765*pH – 0.0136299*Temp (Eq.4)

Table 1. *Physical and chemical parameters measured during MSW compost on days 0, 16, 30, 44, 58,72, 85, 100.*

Parameters	**Days**								
	0	***4***	***16***	***30***	***44***	***58***	***72***	***85***	***100***
Temperature (°C)	24.1	60.3	32.5	29	27.2	28.2	26.8	25	23
pH	6.8	8.1	7.3	7.4	7.25	7.35	7.45	7.3	7.5
Electric conductivity (mS/cm)	1.21	1.94	2.06	1.99	2.1	1.98	1.78	1.8	1.96
Water Content (%)	63.9	50.8	59.1	55.3	51.4	54.6	59.8	55.9	49.6

Discussion

During the composting process, the pH dropped from 8.1 to 7.3, wich may be due to the ammonification and mineralisation of organci matter by the activities of micoorganisms as found by Wong *et al.* (2001). The decline of water content in some cases may be explained by microbial heat generation causing enhanced desiccation (Tiquia and Tam, 2000). The Multiple Regression Analysis, reveal that the parameter that major influence made on the microbial groups mentioned was the temperature (data not shown). In this experiment, the decline of temperature indicated that the compost had gone through the thermofilic stage and approached maturity., the microbial biomass also decreased with composting age, except in the case of actinomycetes.

The increase in bacterial and fungal concentrations evidenced during the mesophilic phase, was influenced fundamentally by temperature and pH. During initial phase of the composting process the substrate is at ambient temperature, the pH is usually slightly acidic and are available easily organic compounds. Mesophilic fungi and bacteria are the dominant active degraders of fresh organic waste materials. Food waste containing vegetable residues often have a low initial pH (4.5-5), which stimulates the proliferation of fungi (Ryckeboer *et al.,* 2003). The high surface/volume ratio of bacteria allows a rapid transfer of soluble substrate into a cell. Nevertheless, actinomycetes are commonly identified as one the main groups responsible for organic matter conversion during latter stages of composting and thermophilic phase (temperatures of 45 to 55°C) according to Chopra (2004) and Velasco *et al.* (2004). High temperatures support degradation of recalcitrant organics such as lignocellulose and elimination of pathogenic and allergenic microorganisms. Actinomycetes compete with others organisms for nutrients and can inhibit microbial growth due to the production of antibiotics, lytic enzymes or even by parasitism. They play an important role in the

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degrading natural polymers process and colonize organic materials after bacteria and fungi have consumed easily degrade fractions (maduration phase); their enzymes enable them to degrade tough debris such as: woody stems, bark or newspaper. The interaction between various functional groups of microorganisms depends on nutrient resources and the biochemical mechanisms of organic and inorganic matter transformation changes (Insam *et al.,* 2002). Microorganisms with high hydrolyzing activities prevailed, they are spore forming (*Bacillus*), mycelial (Streptomyces, *Actinomyces* and fungi) and cellulolytic (essentials fungi). The bacteria *Pseudomonas*, *Azotobacter*, *Azospirillum* use simple organic material formed at hydrolysis and fermentation in aerobic conditions. The redistribution of populations to a degree of dominant and the developmentof stable forms of microorganisms preserve the diversity of species in the structure of community and signify structural-funtional changes.

The genera *Micrococcus, Bacillus, Streptomyces, Actinomyces, Azotobacter, Aspergillus, Penicillium* and *Trichoderma* have been abovementioned by Ryckeboer *et al.* (2003); Velarde *et al.* (2004) and Martínez (2004) in different compost studies. The genera *Ulocladium* sp. has been reported by Seck and Kilbertus (1996) in peanut shells. Several of these genera may find application as potential biocontrol agents are used in the agriculture as fitopathogens biocontrollers agents or as stimulants of the vegetable growth.

Futher non-cultivation studies will be necessary, in order to understand better the behavior of microorganisms and to compare both results. Futhermore, it will be interesting to study some mechanisms of degradation at biochemical levels by the isolated strains.

Acknowledgements. The authors thank Biology Faculty of Havana University for providing laboratory space. We also thank to professor Heribert Insam of the Innsbruck University, Institute of Microbiology, Austria for his valuable book.

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IMPACTS OF GRAZING INTENSITY ON NITROGEN POOLS AND NITROGEN CYCLE IN AN ALPINE MEADOW ON THE EASTERN TIBETAN PLATEAU

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(Received 21th August 2007; accepted 3rd March 2008)

Abstract. Grazers may influence nitrogen (N) pools and alter N inputs and outputs (losses) to the ecosystem in a number of ways. In this study, we evaluated N pools within the plant-soil system (0-30 cm) and soil N transformation under three different grazing intensities by yaks (light: 1.2, moderate: 2.0, and heavy: 2.9 yaks ha^{-1}) in an alpine meadow on the eastern Tibetan Plateau. Total plant and soil N at 0-30 cm depth tended to increase as grazing intensity increasing (plant N: 26.6, 31.4 and 36.7 g m^{-2}; soil N: 905, 939 and 1125 g m^{-2} for light, moderate and heavy grazing, respectively). Soil N transformation rates, such as net N mineralization, gross nitrification, denitrification and N_2O emissions, generally increased under heavy grazing intensity. Results indicate that heavy grazing intensity accelerated the N cycling rates between system components and leaded to increases in plant-soil system N in this alpine meadow.
Keywords: *biomass; grassland; N stocks; soil N transformation; Tibet Plateau*

Introduction

Grasslands occupy approximately one-third of the earth's terrestrial surface and are subject to varying degrees of management by humans [1, 2]. Grazing by livestock is a widely used management tool in grasslands, impacts ecosystem structure and plays a role in regulating nutrient cycling and energy flow [3]. N limits primary production in many terrestrial ecosystems [4]. Determining the role that herbivores play in regulating N storage and cycling in grassland ecosystems is important to understanding not only the grasslands themselves, but also the contribution of grasslands to global nitrogen fluxes.

Grazers can return large amounts of N to the soil through urine and feces, increasing levels of available soil N [3, 5, 6, 7, 8]. Herbivores also increase decomposition rates by reducing C/N ratios of plants [3, 9, 10]. Furthermore, plants often respond to defoliation by decreasing root production [10, 11, 12] that can result in reduced soil C and C/N ratios [10]. Lower C/N ratios in grazed plant material and soils increases net N mineralization by reducing microbial demand for N (i.e., microbial immobilization) during decomposition [10, 13]. However, grazing can also reduce N turnover and availability, as grazers feed selectively on plants with high N content and thus increase the dominance of plant species with low N content, and litter from those species decomposes slowly. Moreover, reducing N availability favors N-poor species because

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they are often better nitrogen competitors [14, 15, 16]. In this case, grazers reduce aboveground productivity and the rate of N cycling. Thus, grazers may have different or even opposite effects on N cycling in different systems and may consequently shift ecosystem productivity in either a positive or negative direction.

The Tibetan Plateau, the largest geo-morphological unit on the Eurasian continent, is an important part of the global terrestrial ecosystem, and one of the major pasture lands in China. Alpine meadows, covering about 35% of the plateau area, are a representative vegetation type and the major grazing land of the region, especially in eastern areas [17]. Long-term overgrazing in the areas has resulted in considerable deterioration and even desertification [18]. However, few data exist quantifying the magnitude and distribution of N stored, and the effects of grazing management on the biogeochemical processes controlling the exchanges of N between the soil and atmosphere.

This study was conducted to examine impacts of three grazing intensities on N pools and soil N cycling in alpine meadow on the eastern Tibetan Plateau. Objectives were to quantify and compare the effects of grazing intensity on (1) plant species composition; (2) above- and belowground biomass; (3) N storage in plants and soil; and (4) soil N transformations, including net N mineralization, net nitrification, gross nitrification, denitrification and N_2O emissions.

Materials and methods

Study site

The study site is approximately 140 ha and located at Hongyuan County, Sichuan Province, China (33°03′N, 102°36′E) and has been previously used as traditional winter pasture (early November to mid-May) by local Tibetan nomads with light grazing intensities [19]. It is 3462 m above sea level, with a continental harsh climate. Annual precipitation averages 752 mm, with about 86% received from May through September. Mean annual temperature is 1.1°C and there is not an absolute frost-free period. The highest monthly mean temperature is 10.9°C in July and the lowest is -10.3°C in January. The dominant species in the whole area was *Clinelymus nutans* and *Roegneria nutans*, accompanied by *Koeleria litwinowii*, *Agrostis schneideri*, *Kobresia setchwanensis* and *Anemone rivularis.* The vegetation covered over 90% [19]. Soils are Mat Cry-gelic Cambisols [20]. Soil organic matter and total N were 61.20 and 3.42 g kg^{-1}, respectively [21].

In 1997, the study site was segregated into several pastures and contracted out to different farmers who established fences to enclose their own pastures. This caused a shift and redistribution of livestock across the study site with grazing intensities varying by farmer, but consistent among years for a given pasture. Three adjacent experimental sites, each with a different grazing intensity, were chosen for study. All the sites had been used as winter pasture with a continuous grazing period from early November to the end of May. Light grazing intensity (LG) was 1.2 yaks ha^{-1} which resulted in 20 to 35% utilization of annual forage production for the 16 ha pasture area, and vegetation was dominated by *Roegneria nutans*, *Deschampsia caespitosa*, and *Elymus nutans*. Moderate grazing intensity was 2.0 yaks ha^{-1}, resulting in 40-50% utilization over the 28 ha pasture, with vegetation dominated by *Kobresia setchwanensis*, *Kobresia pygmaea*, and *Roegneria nutans*. Heavy grazing intensity was 2.9 yaks ha^{-1}, resulting in 60-75% utilization over the 20 ha pasture, with vegetation dominated by *Kobresia pygmaea*, *Kobresia setchwanensis*, and *Potentilla anserina*.

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Field sampling and investigation

In August 2005, five 10 m×10 m plots were randomly selected in each experimental site. Each plot was located at least 12 m from the next nearest replicate. In each plot five 50 × 50 cm quadrates were randomly selected for biomass sampling. Aboveground biomass was clipped to ground level as living biomass and dead biomass (standing dead and litter). Root biomass was measured by collecting 5 soil cores (20 cm in diameter) from depths of 0-30 cm in each plot, which were co-located with the aboveground biomass measurement quadrates. The soil cores (20 cm diameter) were cut into segments corresponding to sampling depths of 0-10, 10-20, and 20-30 cm. These cores were immediately washed over a 1-mm mesh screen to remove soil.

Plant community characteristics were determined from two systematically located transects (50cm×500cm) of ten continuous quadrates (50cm×50cm) in each plot. Plant species were identified and recorded, the total ground cover, species canopy cover, and height determined from 0.25 m^2 quadrates. The frequency of each plant species was calculated for each plot. Importance data for individual species were calculated as averages of their relative abundance in terms of canopy cover, height, and frequency.

Within each plot, composite soil samples consisting of 5 soil cores 7.5 cm in diameter of 30-cm depth were taken from the same five quadrates in which biomass was harvested and root cores were taken. All plant litter was removed from the soil surface before the sampling. Soil samples were segregated into 0-10, 10-20, 20-30 cm increments. Duplicate soil cores were also taken at each sampling quadrate for soil bulk density determination, which were used to convert soil N concentrations (in grams per kilogram) to N mass (in grams per square meter) in the soil.

Rates of in situ net N mineralization and nitrification were evaluated in the field using the buried-bag technique [22]. Five paired soil cores (5 cm diameter, 5 cm depth) were taken from random locations in each plot. In each pair, one soil core was sealed in a gas permeable polyethylene bag and buried at a depth of 5 cm. The other core (initial) was taken adjacent to each buried bag and kept in cooler bags during the transportation to the laboratory prior to freezing for analyses. The buried bags were retrieved after 30 days of incubation and analyzed for NH_4^+-N and NO_3^--N. Net N mineralization was calculated by subtracting initial NH_4^+-N plus NO_3^--N concentrations from final concentrations. Net N nitrification was calculated as the difference in corresponding NO_3^--N concentrations.

We measured gross rates of nitrification, denitrification and N_2O emissions on intact cores using the Barometric Process Separation (BaPS) technique adapted for laboratory incubations [23]. Five intact soil cores (5.6 cm diameter, 4 cm depth) from each plot were collected, which co-located with the other soil cores. The cores were cooled with freezer blocks and returned to the laboratory in insulated boxes for immediate analysis.

Laboratory and statistical analyses

Soil samples intended for N analyses were passed through a 2-mm screen to remove plant crowns, visible roots and root fragments. Samples were air-dried and analyzed for total N by the semi-micro Kjeldahl procedure [24]. Soil extracts were analyzed for NH_4^+-N with the potassium chloride-indophenol blue colorimetric method, and NO_3^--N with calcium sulfate-phenol disulfonic acid method [24].

When soil N transformations were measured, five intact soil samples were directly filled into the BaPS instrument and the system was closed gas tight and incubated at a

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temperature of 11.7°C (average air temperature in August). Determination of soil N processes via the BaPS technique lasted approximately 12 hours [23].

All plant samples were oven-dried for 48 h at 65°C and weighed. Dry samples were then milled and analyzed for N content with the same method as soil sample.

Data were statistically analyzed by one-way analysis of variance (ANOVA) and significant differences were tested by the least significant difference (LSD) at $p<0.05$.

Results

Plant species composition

Difference in plant species composition was observed between the treatments (Table 1).

Table 1. C*omposition of species and their importance value at the three study sites*

Species name	Light grazing	Moderate grazing	Heavy grazing
Roegneria nutans	18.48	8.72	6.06
Elymus nutans	8.54	4.20	3.75
Deschampsia caespitosa	11.43	4.20	
Agrostis schneider	4.18		
Koeleria litwinowii	4.67	5.68	
Kobresia setchwanensis	7.68	17.43	23.32
Kobresia pygmaea	1.70	5.25	12.54
Gueldenstaedtia diversifolia	3.76	3.79	3.34
Oxytropis ochrocephala	2.09	2.78	4.25
Astragalus polycladus		2.09	
Aster alpinus	4.81	6.99	2.78
Saussurea hieracioides		4.60	2.28
Taraxacum maurocarpum	2.20	1.60	1.36
Ligularia virgaurea		1.85	
Leontopodium longifolium	2.60	2.82	6.09
Anemone rivularis	7.33	4.90	3.41
Consolida ajacis	2.32	1.86	
Anemone trullifolia		2.48	2.51
Thalictrum alpinum	1.80	2.26	1.80
Ranunculus brotherusii	1.53	1.74	3.24
Potentilla anserina	1.41	2.13	8.35
Potentilla discolor	1.66		
Geranium phlzowianum	4.19	2.04	1.86
Polygonum viviparum	2.55	5.42	4.11
Stellera chamaejasme	1.32	1.81	3.78
Plantago depressa	1.45	1.15	2.27
Gentiana algida	2.30	1.88	2.88
Total species number	23	25	20
Total cover (%)	89.7	92.6	73.6

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Dominant plant species in the LG site were *Roegneria nutans* (18.48% as importance value), *Deschampsia caespitosa* (11.43%), and *Elymus nutans* (8.54%), and *Kobresia setchwanensis* (7.68%). Major species in the MG site were *Kobresia setchwanensis* (17.43%), *Roegneria nutans* (8.72%), *Aster alpin*us (6.99%), and *Koeleria litwinowii* (5.68%). In the HG site, *Kobresia pygmaea* (12.54%), *Kobresia setchwanensis* (23.32%), *Potentilla anserine* (8.35%), and *Leontopodium franchetii* (6.09%) were most dominant species. Vegetation coverage was highest in the MG site, intermediate in the LG site, and lowest in the HG site.

Above- and belowground biomass

Live, dead and total aboveground biomass was lower in the HG site compared to the other two sites, which did not differ (Table 2).

Across all sites, more than 85% of the total belowground biomass (0-30cm) was in the surface 0-10cm soil depth (Table 2). Total root biomass was lower in the LG site compared to the MG and HG sites, which did not differ. Root/shoot ratio was higher in the HG site, with MG and LG sites similar.

Table 2. *Plant biomass (g m^{-2}) as affected by grazing intensity*

System components	Light grazing	Moderate grazing	Heavy grazing
Above ground			
Live biomass	359.2±53.4a	412.3±65.7a	281.0±39.0b
Dead biomass	162.2±16.7a	177.2±30.1a	111.1±20.1b
Total above ground biomass	521.4±60.4a	589.4±91.4a	392.1±53.6b
Roots			
0-10cm	1523.1±184.1c	2147.0±335.8b	2686.9±449.6a
10-20cm	196.7±52.4	228.5±82.7	152.6±41.2
20-30cm	78.7±14.8	107.0±24.3	84.1±16.0
Total roots	1798.5±179.5b	2482.6±356.2a	2923.6±481.3a
Roots/shoot ratio	3.46±0. 23b	4.27±0.77b	7.51±1.33a
Total plant biomass	2319.9±232.6b	3072.0±397.1a	3315.6±504.7a

Within rows, means ± S.D. Different letters represent statistically significant at $p<0.05$. n=5.

Plant and soil N stocks

Total N storage in the aboveground live and dead biomass was higher in the MG site compared to the LG and HG sites, which did not differ (Table 3). Total root N storage (0-30cm) was higher in the HG site compared to the LG and MG sites, which were similar. Total plant (aboveground + roots) N was greater in the HG site than in the MG and LG sites.

Soil N tended to decrease with increasing of soil depth (Table 3). Soil N storage (0-30cm) was higher in the HG site compared to the LG and MG sites, which did not differ.

Total N was higher in the HG site than the other two grazing intensities, which did not differ (Table 3).

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Table 3. *Total amounts of N stored in plant and soil pools (g m^{-2}) as affected by grazing intensity*

System components	Light grazing	Moderate grazing	Heavy grazing
Above ground			
Live biomass	4.4±0.6b	7.1±1.0a	5.3±0.8b
Dead biomass	1.9±0.3b	2.8±0.6a	1.6±0.2b
Total above ground N	6.3±0.8b	9.9±1.1a	6.9±0.9b
Roots			
0-10cm	18.1±5.2b	19.3±4.6b	28.1±5.6a
10-20cm	1.6±0.4	1.6±0.7	1.1±0.4
20-30cm	0.6±0.1	0.7±0.2	0.6±0.1
Total roots N	20.3±5.1b	21.6±5.0b	29.8±5.8a
Total plant N	26.6±4.7b	31.4±5.0ab	36.7±5.7a
Soil profile			
0-10cm	395.8±43.8b	464.3±93.3ab	541.8±63.2a
10-20cm	289.0±36.1b	289.4±38.9b	359.5±58.6a
20-30cm	219.9±31.0	185.1±30.1	223.7±27.2
Total soil N (0-30cm)	904.7±77.0b	938.8±90.9b	1125.0±81.7a
Total ecosystem N (to 30cm)	931.3±75.4b	970.2±89.2b	1161.7±84.3a

Within rows, means ± S.D. Different letters represent statistically significant at $p<0.05$. n=5.

Soil inorganic N

Soil NO_3^--N content in 0-5cm depth was lower in the MG site than in the LG site or HG sites, which did not differ (Table 4). NH_4^+-N increased with increasing grazing intensity ($p = 0.006$). Total inorganic N (NO_3^--N + NH_4^+-N) was higher in the HG site than in the LG and MG sites, which did not differ.

Table 4. *Mass of soil inorganic N (g m^{-2}) as affected by grazing intensity*

	Light grazing	Moderate grazing	Heavy grazing
NO_3^--N	0.28±0.03ab	0.23±0.02c	0.32±0.05a
NH_4^+-N	0.37±0.06c	0.50±0.08 b	0.64±0.13 a
Total inorganic N	0.65±0.09 b	0.73±0.09 b	0.96±0.18 a

Within rows, means ± S.D. Different letters represent statistically significant at $p<0.05$. n=5.

Soil N transformations

The HG site had higher net N mineralization, gross nitrification and denitrification rate than the LG site, with the MG site intermediate (Table 5). Net nitrification was higher at the HG compared to the MG site ($p = 0.080$). N_2O flux rate was higher in the HG site compared to the LG and MG sites, with no difference between the LG and MG sites.

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Table 5. *Soil N transformation rates (mg N m^{-2} d^{-1}) as effected by grazing intensity*

	Light grazing	Moderate grazing	Heavy grazing
Net N mineralization	1.83±0.73b	2.54±0.97ab	4.10±1.80a
Net N nitrification	5.97±1.42ab	3.42±0.99b	7.41±3.00a
Gross N nitrification	226.4±57.9b	272.4±83.2ab	344.3±30.2a
Denitrification	23.79±4.56b	26.79±6.19ab	36.56±10.16a
N_2O emission rate	2.98±0.50b	3.15±0.72b	4.62±1.47a

Within rows, means±S.D. Different letters represent statistically significant at $p<0.05$. n=5.

Discussion

The magnitude of impact that livestock grazing may have on a plant community is dependent upon intensity of grazing. In contrast to grazing at a light or moderate grazing intensity, grazing at heavy intensity has tended to decrease the numbers of grasses such as *Roegneria nutans* and *Deschampsia caespitosa* and increased the numbers of sedges such as *Kobresia setchwanensis* and *K. pygmaea*, which is good tolerant to be grazed, specially for yaks [25]. Heavy grazing also markedly reduced vegetation cover compared to light grazing and moderate grazing. This has an important implication for grassland management because vegetation cover is often used to assess spatial extent and degree of desertification [26].

Plant biomass is an important measure of ecosystem functioning for alpine meadows. After eight years grazing with different intensity, live, dead and total aboveground biomass was lower with heavy grazing compared to light or moderate grazing intensity. The reason for these results was that the dominator of HG community shifted from grasses-*Roegneria nutans* and *Deschampsia caespitosa* into sedges-*Kobresia pygmaea* and *K. setchwanensis*, which are small and good tolerant to be grazed [24]. Also, as our results demonstrated, a larger proportion of total production was allocated to the belowground biomass with heavy grazing [27]. Aboveground biomass decreased under heavy grazing intensity indicated that the winter forage supply for this region reduced and accordingly the pressure on native grassland productivity increased.

Root biomass responses to grazing are ambiguous. Milchunas and Lauenroth [28], Turner et al. [29], and Frank et al. [30] found mostly no changes, or increases, of root biomass as a function of grazing intensity. Our results suggested belowground biomass was lowest in the LG site and higher in both the MG and HG sites. This can be explained that heavy grazing induced more *Kobresia pygmaea* and *K. setchwanensis*, which have larger root system than that of *Roegneria nutans* and *Deschampsia caespitosa* [31]. This change was reflected in the higher root to shoot biomass ratio under the heavy grazing treatment compared to light grazing treatment. Biomass allocation ratio to root increasing is an adaptive response of plant to grazing. High proportion of root biomass in the total biomass can increase the capacity to tolerate environmental stresses and external disturbances, which is favorable for grassland restoration [32].

N storage of plants may depend on soil nitrogen availability [33, 34]. Our results supported the hypotheses because higher soil N availability occurred when grazing intensity increased. Moreover, greater availability of labile N in the soil on higher grazing intensity sites could increase plant uptake rate of N [30, 35]. In addition, a higher root biomass by increasing grazing intensity was contribution to plant N uptake

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[36]. Although high N storage in belowground biomass is common in grasslands [37], the effects of grazing intensity on this pattern were different (i.e., HG amplified this pattern compared to MG and LG). Those results suggested that grazing intensity changed the above- and belowground allocation of N within plant. Belowground N allocation may allow compensatory responses to grazers [29], and might also facilitate the recovery of vegetation after natural disturbance [38].

Soil N storage was higher under the heavy grazing intensity compared to the light grazing intensity, consistent with results for the northern mixed-grass prairie reported by Dormaar et al. [39] and Manley et al. [40]. The higher soil N under grazing intensity could be due to differences in root biomass between the treatments. Schuman et al. [41] and Hibbard et al. [42] reported larger root biomass can contribute more C and N to soil in northern mixed-grass and semi-arid grassland, respectively. In addition, the feces and urine from grazers are also important N source of soil [5, 8].

Heavy grazing had consistently higher soil N transformations than the light grazing in this alpine meadow ecosystem. These findings were more in line with corroborate those of Olofsson et al. [15] and Le Roux et al. [43] who all found that increasing grazing intensity increased soil N cycling rates. There were several mechanisms that could explain the increases of soil N processes under heavy grazing intensity. The N added by grazers in excrement is in forms available to plants and soil microbes [3, 5, 6, 7]. The increased litter decomposition resulted from grazer trampling. Faster decomposition means that less nitrogen was tied up in litter and nitrogen may cycle through the ecosystem more quickly [3, 40]. Another mechanism through which yaks may affect soil N transformations was through altering soil microclimate, such as soil temperature or soil water content [44]. The higher soil N mineralization and nitrification can be responsible for the higher soil inorganic N content under heavy grazing intensity [45, 46]. In comparison, net nitrification did not show a similar pattern as other soil N transformation rates, the lowest value occurred at the MG site. This might be attributed to the excess consumption of NO_3^--N by the faster accumulating of aboveground biomass at the MG site. Gross nitrification rates were 37-80 times the net nitrification rates, which was similar to the result reported by Sun et al. [47] in a subalpine meadow soil. Higher soil N transformations under heavy grazing intensity also implied that a potential for leaching losses of N as NO_3^- and N_2O losses, via nitrification or denitrification [48].

Conclusion

Although heavy grazing intensity resulted in higher levels of plant and soil N, it decreased vegetation coverage and aboveground biomass, which are undesirable for livestock production and sustainable grassland development. What is more, heavy grazing could also introduce potential N loss via increasing NO_3^- leaching to ground water and N_2O emission to the atmosphere. Grazing at light to moderate intensity resulted in the plant communities dominated by forage grasses with high aboveground biomass productivity and N content. The alpine meadow ecosystems in Tibetan Plateau are very fragile and evolved under grazing by large herbivores; therefore, without an appropriate level of grazing in a long term perspective on an ecological timescale, deterioration of the plant-soil system, and possible declines in soil N, are indicated.

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Acknowledgements. This study was financially supported by the Key project of the National "10th Five-year Plan" of China (2001BA606A-05), the National Natural Science Foundation of China (90511003), the Important Orientation Project of the Chinese Academy of Sciences (KSCXI-07, KSCX2-01-09), the Project of "100 Distinguished Yong Scientists" of the Chinese Academy of Sciences (2004) and the Youth Sci & Tech Foundation of Sichuan (03ZQ026-043), China. The authors thank Prof. T. Wei for his help on identification of plant species, and Mr. J. Chen and Mrs. J. Pei for their help in the laboratory work.

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REVIEW ON REMOVAL OF HEAVY METALS FROM ACID MINE DRAINAGE

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(Received 29th August 2007; accepted 24th June 2008)

Abstract. Acid mine drainage (AMD) is one of the most significant environmental challenges facing the mining industry worldwide.This article summarizes the literature on the removal of heavy metals from acid mine drainage wastewater by various techniques. Investigation has been carried out across the world in order to control and decrease the level of heavy metals as pollutants from acid mine drainage wastewater.
Keywords: *Treatment, effects, metals, coal mine waste.*

Introduction

Abandoned Mine Drainage (also referred as AMD) is water that has become contaminated as a result of passage through a physical environment created by coal mining activities of the past. This contamination can occur in the underground voids created by deep or underground mining or it can occur by water passing through coal mining refuse left on the surface.

The vast majority of this type of pollution results from old mining operations that have simply been abandoned after the coal was extracted. In some cases, the AMD is from very old mining operations dating from the turn of the 20th century. Prior to 1977, the laws governing coal mining operations were less stringent concerning their environmental impacts. It was a common practice to simply abandon mining operations following the exhaustion of the coal reserve, and then declare bankruptcy. This allowed the mining operators to walk away from liabilities, including environmental devastation.

The nature of AMD contamination varies greatly from site to site, as its formation is dependent on different factors. AMD often lowers water quality and impairs aquatic life, and is most often characterized by one or more of the four major components:

- Low pH (high acidity), i.e., acid mine drainage
- High metal concentrations (iron is the most common)
- Elevated sulfate level
- Excessive suspended solids and/or siltation

The majority of AMD problems result from surface water contact with the unreclaimed waste rock and other earthen materials or from the seepage or drainage of ground water which has contacted the coal or rock strata remaining in an underground mine. If the water becomes acidic, it is referred to as "acid mine drainage". Acid is a

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contaminant of primary concern since it can leach toxic concentrations of metals from rocks at mine sites.

Acids in streams are a problem because they can corrode metal pipes and structures, break down concrete, and kill or stunt plants and other aquatic life-forms. Acidic surface waters or runoff can also break down metallic compounds of iron, sulfur, manganese, and aluminum found in nearby rock or earthen waste piles.

Acid solutions form when surface or ground water comes into contact with acidic material, mostly pyrite, commonly found in mine rocks, earthen refuse piles, or underground mine works and/or auger holes. The iron-sulfide mineral pyrite is often found near subsurface coal seams along with compounds containing manganese, aluminum, and other metals. In the presence of oxygen, ordinary rain water or ground water can react with the sulfur to form sulfuric acid.

Acid concentrations in AMD can reach levels that are more than 10,000 times higher than neutral waters, presenting a powerful leaching agent that can dissolve significant amounts of metal compounds and leach additional acid from rocks and earthen wastes commonly found at most mine sites.

Layers of rock and earth above the coal removed during mining commonly contain traces of iron, manganese, and aluminum and can also contain other heavy metals. These metals can be dissolved from mining sites through the action of acid runoff, as described above, or can be washed into streams as sediment. Many metals, though common, can be toxic to fish and other aquatic organisms when they are present in high dissolved concentrations. Dissolved iron and iron precipitate, for example, can kill the aquatic biota that fish feed on, thus reducing the overall fish population. Iron precipitate can also clog the gill structures of fish which will eventually lead to their death as well. In addition, precipitation of iron in the stream channel can also wipe out the aquatic food chains and adversely affect fish populations.

As pyrite wastes are chemically broken down, a sulfate compound is produced in runoff waters. Sulfates can bond with water molecules to form sulfuric acid or can attach to calcium atoms to form gypsum sludge. Elevated sulfate levels are often found in AMD discharges.

Most people think contaminated AMD results from chemical reactions in streams, but a significant threat to water quality and aquatic organisms comes from eroding soils at abandoned mining sites. Tiny fly nymphs, insect larvae, and other organisms that form the base of aquatic food chains can be wiped out by heavy accumulations of soil and mine waste particles that wash into streams after rain events. Suspended silt particles can clog the gills of fish and smother eggs on the stream bottom. Streams and rivers muddied by silt and other suspended solids also mean higher costs at municipal and industrial water treatment plants and accelerated sedimentation in reservoirs.

Acid mine drainage (AMD) is a common problem at thousands of abandoned mine sites all over the world. The combination of low pH and high concentrations of metals associated with mine drainage can have severe toxicological effects on aquatic ecosystems. Acute exposure to high concentrations of metals can kill organisms directly, while long-term exposure to lower can cause mortality or other effects such as stunted growth, lower reproduction rates, deformities and lesions (Lewis and Clark, 1996).

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AMD is caused by the weathering of minerals such as iron disulphide (FeS2), commonly known as pyrite, by water and oxygen (Singer and Stumm, 1970).When pyrite is exposed to water and oxygen, oxidation and hydrolysis reactions produce sulfuric acid (H2SO4) and free hydrogen ions (H+), acidifying the water. Lower acidities allow other metals which are associated with mining, such as cadmium, copper, lead and zinc, to enter the solution phase and be transported from the system (Stumm *et al.*, 1996). These metals precipitate from the water column as pH increases, however the pH at which there is a potential for complete precipitation varies for different metals. For example, zinc precipitates at a minimum pH of 8.4 (Stumm *et al.*, 1996). Transport of zinc can occur over long distances, since the pH of many streams is less than 8.0. The rate of the AMD weathering reactions is limited by the surface area of ferrous iron exposed to weathering (Gray, 1997). The process of extracting ore from underground veins alters hydrologic processes by creating new pathways. As a result of mining activities, water and oxygen are able to infiltrate areas of high metal-sulfide mineral content at an increased rate, accelerating the natural acid-producing weathering processes.

Heavy metal pollution

There are many elements presents on the earth's crust. H, C, N, O, Na, Mg, P, S, Cl, K, Ca, constitute 99.9% of all living matter. In addition 14 elements viz. B, F, Si, V, Cr, Mn, Fe, Co, Cu, Sc, Mo, Sn and l are the essential elements. Metals like Hg, Pb, Cd, As, Cr, Zn, Cu, Mn which are not essential but when present in aquatic environment are hazardous.

Although heavy metals are natural components of the environment but when their concentrations increase more than normal levels they become potentially hazardous. The examples include diseases like itai-itai disease, mancigata (Diospyros melanoxylon) and lead poisoning. Hence the task of the environmental scientist is to regulate their influx into the environment in biologically assumable forms and acceptable levels.

There are two broad classes of methodologies used to treat Acid Mine Drainage:

Passive Treatment: natural chemical and biological reactions occuring in a controlled microbiological-chemical reactor without powered mechanical assistance (most of the time).

Active Treatment: mechanical addition of alkaline chemicals to raise pH and precipitate metals.

This paper is a comparative study of these methods to remove heavy metals from Acid Mine Drainage.

B-W. Zhang et al. (1993) prepared amidoxime-containing modified starch by reaction of acrylonitrile with cross linked starch, catalyzed by ceric ions, and subsequent derivatization with free hydroxylamine in methanol. The early investigations of this new cation exchange material revealed that the maximum capacities of the modified starch for the heavy metal ions Cu 2+, Hg 2+, Pb 2+ and Zn 2+ were 1.86, 0.59, 0.52 and 0.37 mmol g-1 (dry weight), respectively. At pH 5, the order of decreasing selectivity of the cation exchanger for the various metal ions was: Hg2+> Cu2+> pb2+> Zn2+> Ni2+> Cr 3+. The product could be reused after

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regeneration with diluted hydrochloric acid. The high exchange capacity for copper was fully examined because of the possible industrial application.

Yi-Min Gao et al. (1995) identified and studied a new hybrid iron-rich inorganic sorbent in relation with heavy metal removals in fixed-bed processes for influent pH as low as 3.5. Every single particle of this new hybrid sorbent essentially contains ferrihydrite along with a crystalline silicate phase, akermanite, in close proximity (in the order of 100 A^{O}) to one another. Akermanite has a unique ability to produce hydroxyl ions through incongruent hydrolysis reactions without being washed out from the fixed bed. The simultaneous presence of akermanite and ferrihydrite in a single particle has a synergistic effect on the sorption process: while akermanite helps neutralize aqueous-phase hydrogen ions (thus enhancing sorption capacity of ferrihydrites), neighboring sorption sites in ferrihydrites quickly remove dissolved heavy metals, thus avoiding precipitation. Equally important, the hybrid sorbent can be regenerated with any amine/ammoniacal solution and reused for multiple number of cycles. Some precipitations may occur within the column at relatively high influent concentrations of heavy metals (around 50 mg/1) or due to chromatographic effect. Such precipitates are, however, amenable to removals by conventional backwashing.

Jack Z. Xie et al. (1996) prepared Biosorbents and chemically modified them, then compared with commercial ion-exchange resins to determine their metal-binding capacity, the range of metals bound, the effects of pH, temperature, contact time, interference by common salts and the effect of multiple cycles of metal binding and elution. Biosorbents were prepared from microorganisms isolated from pristine and metal impacted (acid mine drainage) environments and included heterotrophs, methanotrophs, algae, sulfate reducers, and exopolysaccharide- producing cultures. The chemical modifications examined included encapsulation in polysulfone resin, acid, alkali, carbon disulfide, phosphorus oxychloride, anhydrous formamide, sodium thiosulfate, sodium chloroacetic acid, and phenysulfonate treatments. A culture isolated from an acid mine drainage impacted site, IGTM17, produced biosorbent material with about three-fold higher metal-binding capacity than other biosorbents examined in this study.

Teruyuki Umita (1996) studied the Drainage from sulfur mines contains a high concentration of ferrous iron. The mechanism of acid mine drainage formation was briefly explained. As a case study, successful measures taken at the abandoned Matsuo mine, Iwate Prefecture, Japan, for preventing the pollution in receiving rivers was presented in this paper. The measures consisted of the construction works against pollution sources and the construction of a drainage treatment plant in which *Thiobacillus ferrooxidans* oxidizes ferrous iron under a low pH condition, and produced ferric iron is removed by sedimentation. Then, a laboratory-scale fluidized bed reactor using anion exchange resin as attaching material for the bacteria was examined in order to improve the efficiency of biological oxidation of ferrous iron. More than 90% of oxidation had been maintained for 2 months at 1 h of HRT, which suggests that the size of the oxidation tank could be reduced.

P.D. Rose et al. (1998) investigated that acid mine drainage pollution may be associated with large water volume flows and exceptionally long periods of time over which the drainage may require treatment. While the use and role of sulfate reducing bacteria has been demonstrated in active treatment systems for acid mine drainage remediation, reactor size requirement and the cost and availability of the carbon and electron donor source are factors which constrain process development. Little attention

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has focused on the use of waste stabilization pounding processes for acid mine drainage treatment. Wastewater pounding is a mature technology for the treatment of large water volumes and its use as a basis for appropriate reactor design for acid mine drainage treatment is described including high rates of sulfate reduction and the precipitation of metal sulfides. Together with the co-disposal of organic wastes, algal biomass is generated as an independent carbon source for SRB production. Treatment of tannery effluent in a custom-designed high rate algal pounding process, and its use as a carbon source in the generation and precipitation of metal sulfides has been demonstrated through piloting to the implementation of a full-scale process. The treatment of both mine drainage and zinc refinery wastewaters is reported. A complementary role for micro algal production in the generation of alkalinity and bioadsorptive removal of metals has been utilized and an Integrated 'Algal Sufhate Reducing Pounding Process for the Treatment of Acidic and Metal Wastewaters' (ASPAM) has been described.

Young-Wook Cheong et al. (1998) designed, operated and then evaluated a pilot reactor system for testing acid mine drainage treatment. at the Dalsung mine, for operating problems. Field analyses of pH and Eh (redox potential) were made. In the laboratory, analyses were made of Cd, Pb, As, Zn, Cu, Fe, Al and Mn. The reactor, containing a mixture of rice stalks, cow manure, and limestone, initially showed decreases of 98% Cu, 100% Zn, 99% Fe, 100% Cd, 97% Al, 61% Mn and 100% Pb when the effluents from the reactor had a pH of 6 and an Eh of about 300 mV. However, as time passed, the Eh rose and the amount of metals removed decreased, except Al. This indicated that maintaining reducing conditions was very important for continued metal removal. During the operating period, there were some problems such as a volume change in the substrate within the reactor and scaling on pipes. These problems appeared to reduce the flow of mine drainage in pipes and the reactor over time.

Phillip Elliott et al. (1998) developed a system for the remediation of acid mine drainage using sulfate-reducing bacteria. An up flow porous medium bioreactor was inoculated with sulfate-reducing bacteria (SRB) and operated under acidic conditions. The reactor was operated under continuous flow and was shown to be capable of sulfate reduction at pH 4.5, 4.0, 3.5 and 3.25 in a medium containing 16.1 mM sodium lactate. This contrasted a previously published work which showed that, at pH 3.8, organic acid concentrations higher than 5 mM completely inhibited biological sulfate reduction. At pH 3.25 the reactor removed 38.3% of influent sulfate and raised the pH of the medium to 5.82. The lactate carbon source was exhausted under these conditions. At pH 3.0, sulfide production was below detectable levels, and sulfate removal fell to 14.4%. However, viable SRB were recovered from the column after 21 days of operation at pH 3.0, indicating that SRB are able to withstand pH 3.0 for extended periods. As a conclusion, we can state that an SRB process would be a viable method of remediating AMD.

Carl S. Kirby et al. (1998) developed a gravity-fed, battery-powered, portable continuously-stirred tank reactor to directly measure aqueous reaction rates in the field. Dye and tracer experiments indicate the reactor is well-mixed. Rates of Fe2+ oxidation at untreated and passively treated coal mine drainage sites in Pennsylvania were measured under ambient conditions and with the addition of either O2 gas or NaOH solutions. Uncertainties in rates ranged from 70% near the lower limit of measurement to as little as 1% at higher rates of reaction. Multiple linear regressions showed no universal correlations of rates to Fe2+, dissolved O2, and pH (Thiobacillus populations

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were not measured), although data for two more acidic sites were found to fit well for the model log rate= log K+ a log [Fe2+]+b log [OH–] +c log [O2]. Field rates of Fe oxidation from this and other studies vary by 4 orders of magnitude. A model using the ambient field rate of Fe oxidation from this study successfully reproduced independently-measured Fe2+ concentrations observed in a passive wetland treatment facility.

T. K. Tsukamoto et al. (1999) revealed that the Sulfate reducing passive bioreactors are increasingly being used to remove metals and raise the pH of acidic waste streams from abandoned mines. These systems commonly use a variety of organic substrates (i.e. manure, wood chips) for sulfate reduction. The effectiveness of these systems decreases as easily accessible reducing equivalents are consumed in the substrate through microbial activity. Using column studies at room temperature (23-26°C), we investigated the addition of lactate and methanol to a depleted manure substrate as a method to reactivate a bioreactor that had lost >95% of sulfate reduction activity. A preliminary experiment compared sulfate removal in gravity fed, flow through bioreactors in which similar masses of each substrate were added to the influent solution. Addition of 148 mg/l lactate resulted in a 69% reduction in sulfate concentration from 300 to 92 mg/l, while addition of 144 mg/l methanol resulted in an 88% reduction in sulfate concentration from 300 to 36 mg/l. Because methanol was found to be an effective sulfate reducing substrate, it was chosen for further experiments due to its inherent physical properties (cost, low freezing point and low viscosity liquid) that make it a superior substrate for remote, high elevation sites where freezing temperatures would hamper the use of aqueous solutions. In these column studies, water containing sulfate and ferrous iron was gravity-fed through the bioreactor columns, along with predetermined methanol concentrations containing reducing equivalents to remove 54% of the sulfate. Following an acclimation period for the columns, sulfate concentrations were reduced from of 900 mg/l in the influent to 454 mg/l in the effluent that reflects a 93% efficiency of electrons from the donor to the terminal electron acceptor. Iron concentrations were reduced from 100 to 2 mg/l and the pH increased nearly 2 units.

E.I. Robbins et al. (1999) evaluated the processes affecting neutralization of acidic coal mine drainage within 'anoxic' limestone drains (ALDs). Influents had pH 3.5 and 2 mg/l dissolved oxygen. Even though effluents were near neutral (pH 6 and alkalinity, acidity), two of the four ALDs were failing due to clogging. Mineral-saturation indices indicated the potential for dissolution of calcite and gypsum, and precipitation of Al31 and Fe31 compounds. Cleavage mounts of calcite and gypsum that were suspended within the ALDs and later examined microscopically showed dissolution features despite coatings by numerous bacteria, biofilms, and Fe–Al–Si precipitates. In the drain exhibiting the greatest flow reduction, Al-hydroxysulfates had accumulated on limestone surfaces and calcite etch points, thus causing the decline in transmissivity and dissolution. Therefore, where Al loadings are high and flow rates are low, a pre-treatment step is indicated to promote Al removal before diverting acidic mine water into alkalinity-producing materials.

D. Feng et al. (2000) characterized and treated Acid mine water from a South African gold mine by the precipitation of heavy metals with lime and sulfides, followed by ion exchange. The novelty of the proposed process lies in the use of carrier magnetic materials for more effective separation of water and solids, as well as the oxidation pretreatment that is also used to sterilize the water. The process can generate vet), pure

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water from acid mine water with a great flexibility and an acceptable cost. The oxidation anti precipitation of heavy metals with lime and subsequent sulfide-carrier magnetic separation appeared to be particularly suitable for the removal of heavy metal ions from the effluent of the particular gold mine that was investigated. The cation exchange resin IR120 can be used to reduce the salinity of the effluent of mine water after removal of heavy metals by precipitation. Low cost sulfuric acid can be used as the cation resin regenerator. The anion exchange resin A375 could reduce the anions (sulfate, chloride, bromide and fluoride) to acceptably low levels in the mine water after precipitation of heavy metals. A combination of sodium hydroxide and saturated lime solution can be used as the anion resin regenerator. A mixture of acidic gypsum from the cation elution section and alkaline gypsum from the anion elution section could generate high quality gypsum as by-product, which could be sold as a valuable raw material to the gypsum industry, to offset process cost. Although these experiments were conducted on the acid mine water of a specific mine, the process could be extended to other mine waters contaminated with heavy metals and high salinities.

Vivek Utgikar et al. (2000) states that biosorption is a potentially attractive technology for treatment of acid mine drainage for separation/recovery of metal ions and mitigation of their toxicity to sulfate reducing bacteria. This study describes the equilibrium biosorption of Zn(II) and Cu(II) by nonviable activated sludge in a packed column adsorber. The Zn(II) uptake capacity of unconditioned sludge (not subjected to processing other than drying) was found to decrease in repeated adsorption-desorption cycles, declining by a factor greater than 20 from cycle 1 to cycle 6. Equilibrium uptake of metals by dried sludge conditioned by exposure to deionized water at a pH corresponding to that of the feed solution showed a strong pH dependence and was modeled using the Langmuir adsorption isotherm. Equilibrium metal uptakes from solutions containing single metal ion were 2.5 mg g(dry biomass)$^{-1}$ and 3.4 mg g(dry biomass)$^{-1}$ for Zn(II), and 1.9 mg g(dry biomass) $^{-1}$ and 5.9 mg g(dry biomass)$^{-1}$ for Cu(II) at pH 3.0 and 3.8, respectively. Equilibrium uptakes from binary mixtures were 30% lower than single component solution uptakes for both metals, indicating some competition between the two metals. No hysteresis was detected between adsorption and desorption equilibriums. Anion concentration and pH measurements indicated that simultaneous sorption of metal cation and sulfate anion was probably occurring at pH 3.0, while proton exchange predominated at pH 3.8. Results of the study point to the usefulness of non-viable activated sludge as a biosorbent for recovery/separation of metal ions from acid mine drainages.

Seop Chang et al. (2000) tested organic waste materials as the electron donors for sulfate reducers in treating acid mine drainage. They are oak chips (OC), spent oak from shiitake farms (SOS), spent mushroom compost (SMC), sludge from a wastepaper recycling plant (SWP) and organic-rich soil (ORS). The materials were placed in column reactors, which were inoculated by anaerobic digester fluid. The number of sulfate-reducers increased to 108±109 cells/ml in 2 weeks in the reactors. The reactors were continuously fed with simulated mine drainage for 35 weeks to determine sulfate reduction and metal removal. SOS, SMC and SWP served well than electron donors than OC and ORS. Metal removal efficiency of the reactor with OC was lower than those with SOS, SMC and SWP at the beginning, but became comparable at a later stage. The solvent extractable of OC inhibited the growth of sulfate reducers. Cellulose polysaccharides were the main components of the waste materials consumed in the reactors.

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Matthew M. Matlock et al. (2002) developed 1,3-benzenediamidoethanethiol dianion (BDET, known commercially as MetX) to selectively and irreversibly bind soft heavy metals from aqueous solution. In the present study BDET was found to remove >90% of several toxic or problematic metals from AMD samples taken from an abandoned mine in Pikeville, Kentucky. The concentrations of metals such as iron, may be reduced at pH 4.5 from 194 ppm to below 0.009 ppm. The formation of stoichiomietric BDET-metal precipitates in this process was confirmed using X-ray powder diffraction (XRD), proton nuclear magnetic resonance (1H NMR), and infrared spectroscopy (IR).

Hyun-Joon La1 et al. (2003) studied three laboratory-scales, up flow anaerobic reactors were operated for about 250 d to determine the effect of activated granular sludge with high density of sulfate reducing bacteria in the treatment of artificial acid mine drainage. Sulfate reducing bacteria in the granular sludge taken from the up flow anaerobic sludge blanket reactor were 1– 2 × 106 c.f.u. $g^{-}1$, which is at least 10 times higher than that of organic substrates such as cow manure and oak compost. The reactors with granular sludge effectively removed over 99% of heavy metals, such as Fe, Al, Cu, and Cd during the experiment. This result suggests a feasibility of the application of granular sludge as a source of sulfate reducing bacteria for the treatment of acid mine drainage.

M.M.G. Chartrand et al. (2003) reveled that acid mine drainage (AMD), which has long been a significant environmental problem, results from the microbial oxidation of iron pyrite in the presence of water and air, affording an acidic solution that contains toxic metal ions. Electrochemical treatment of AMD offers possible advantages in terms of operating costs and the opportunity to recover metals, along with cathodic reduction of protons to elemental hydrogen. This work describes the electrolysis of synthetic AMD solutions containing iron, copper and nickel and mixtures of these metals using a flow-through cell divided with an ion exchange membrane. Iron was successfully removed from a synthetic AMD solution composed of FeSO 4/H2SO4 via Fe(OH)3 precipitation outside the electrochemical cell by sparging the electrolyzed catholyte with air. The work was extended to acidic solutions of Fe 2þ, Cu2 þ, and Ni2þ, both singly and in combination, and to an authentic AMD sample containing principally iron and nickel.

T.K. Tsukamoto et al. (2004) states that the lifetime of traditional sulfate-reducing bacteria (SRB) bioreactors that utilize a source of reducing equivalents contained within the matrix (e.g. manure) is limited by the amount of readily available reducing equivalents within that matrix. In order to extend bioreactor lifetime indefinitely, the addition of known concentrations of alternative reducing equivalents (methanol and ethanol) to a depleted matrix was tested at low pH and low temperatures. Following acclimation, up to 100% efficiencies of reducing equivalents were directed toward sulfate reduction. Alcohol was added in stoichiometric concentrations to remove 50% of the added sulfate (900 mg/L), producing sufficient sulfide to precipitate all of the iron from solution. An average of 42% of the sulfate was removed following acclimation, reflecting 84% efficiency. An average of 93% of the iron was removed (93 mg/L). Bacteria acclimated to ethanol more rapidly than methanol, although both alcohols were effective as carbon sources. Efficient treatment was observed at the lowest temperatures (6°C) and lowest pHs (pH=2.5) tested. The use of ethanol-fed, highly permeable bioreactor matrices of wood chip, pulverized plastic and rock was also examined to determine which of these porous matrices could be implemented in a field bioreactor. Results indicated that >95% of the 100 mg/L iron added was removed by all matrices.

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Sufficient reducing equivalents were added to remove 450 mg/L of sulfate, wood and rock matrices removed B350 mg/L plastic removed B225 mg/L. A study comparing rock size indicated that small rocks removed iron and sulfate more efficiently than medium- and large-size rocks. The results suggest that wood and rock in conjunction with ethanol are viable alternatives to traditional bioreactor matrices. These findings have direct application to semi-passive sustained operation of SRB bioreactors for treatment of acidic drainage at remote sites.

E. Erdem et al. (2004) studied the adsorption behavior of natural (clinoptilolite) zeolites with respect to Co2+, Cu2+, Zn2+, and Mn2+ in order to consider its application to purity metal finishing wastewaters. The batch method has been employed, using metal concentrations in solution ranging from 100 to 400 mg/l. The percentage adsorption and distribution coefficients (*K*d) were determined for the adsorption system as a function of sorbate concentration. In the ion exchange evaluation part of the study, it is determined that in every concentration range, adsorption ratios of clinoptilolite metal cations match to Langmuir, Freundlich, and Dubinin–Kaganer–Radushkevich (DKR) adsorption isotherm data, adding to that every cation exchange capacity metals has been calculated. It was found that the adsorption phenomena depend on charge density and hydrated ion diameter. According to the equilibrium studies, the selectivity sequence can be given as

Co2+ > Cu2+ > Zn2+ > Mn2+. These results show that natural zeolites hold great potential to remove cationic heavy metal species from industrial wastewater.

M. C. Costa et al. (2005) found that the acid mine drainage (AMD) is a serious environmental problem resulting from extensive sulfide mining activities. The old copper mine of S. Domingos in Southeast Portugal is an example of such a situation. The abandoned open-pit from the mining operations resulted in the creation of a large pit lake with acidic water (pH-2) and high contents of sulfate and heavy metals. Sulfate reducing biological processes have been studied as a remediation technology for this problem. A new application based on a simple and semi-continuous process for the treatment of S. Domingos AMD has been presented herein. Experiments using bench scale fixed-bed column bioreactors were carried out to evaluate the efficiency of the process. Sewage, anaerobic sludge and soil from the mining area were tested as solid matrices and/or inocula, as well as sources of complex organic substrates. The addition of lactose as a supplementary carbon source, easily available at zero cost or at negative cost in the effluents of the local cheese industries, was also tested. The data obtained indicate that it is possible to use the matrices tested for the production of sulfide by sulfate reduction, and that the regular addition of lactose is effective. Results showed that the process is efficient for the precipitation of the main dissolved metals, for the reduction in the sulfate content and, most importantly, for the neutralization of the AMD. Moreover, the use of soil as solid support also showed the possibility of using this process for the decontamination of both waters and soils.

Louis R. Bernier (2005) assessed **t**he efficiency of serpentinite as an alternative alkalinity generating material for the passive treatment of acid mine drainage (AMD). Three series of batch experiments were designed for the passive treatment of a low pH (1.6) AMD synthetic solution containing 2,500 ppm Fe2+, 6,600 ppm SO4 2), 10.5 ppm Al, 15 ppm Ni, and traces of Cr, Mn and Cu. The influencing factors studied were: the effect of water/rock ratio, residence time, type of the alkalinity generating material (dolomite, magnesite, marble, serpentinite), and nature of the system (open vs. closed cells). The variations in solution chemistry observed in the open cells indicate that a

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lower water/rock ratio (0.33 ml/g) was the most efficient for metals removal. The optimal residence time in open cells was 24 h to reach the higher pH values. In the closed cells laboratory setup, synthetic AMD was placed in contact with the various alkaline materials for three different contact times (24, 48, 72 h). The optimal pH was reached after 48 h and did not change appreciably for longer contact time, and the best results for metal removals were obtained with marble and serpentinite. Single treatment efficiency was compared with a successive treatment approach. The most promising results were obtained with a five step treatment: (1) pre-treatment in a closed cell using serpentinite, (2) aeration and settling, (3) treatment in an open cell using marble, (4) final aeration and settling, and (5) filtration with a coarse silica sand. With this configuration, the final pH was 6.5 and pronounced metals depletion was achieved (100% for Al, 99.95% for Fe, 85.7% for Ni).

Xinchao Wei et al. (2005) studied that the large volumes of sludge produced through the active treatment of acid mine drainage (AMD) require further processing and final disposal. AMD sludge typically contains a heterogeneous mixture of iron, aluminum, magnesium, and calcium oxides/hydroxides that are of little to no practical value. However, purified iron and aluminum hydroxides have potential commercial value. Based on the solubility of the major dissolved metals, a two-step selective precipitation process was developed to recover high purity iron and aluminum as separate hydroxide products through the manipulation of current AMD treatment operations. The recommended pH for iron precipitation was pH 3.5–4.0 with precipitate purity >93.4% and iron recovery >98.6%. AMD water after iron removal was used as source water for aluminum recovery. Aluminum precipitated best at pH 6.0–7.0 with aluminum recovery >97.2% and precipitate purity >92.1%.

Jacco L. Huisman et al. (2006) investigated that one of the best available technologies for the removal of metals from water is in the form of metal sulfides. Metal removal by sulfide precipitation is a well-known process that is characterized by compact residues and very high removal efficiencies. Compared to neutralization alone the sludge volume is 6 to 10 times lower and the toxic metals are removed to a 0.01–1 ppm level. Furthermore, selective metal precipitation is possible, allowing for separate recovery of valuable metals like copper, nickel, cobalt and zinc from nuisance metals like arsenic and antimony. However, the cost of reagent (NaHS or H2S gas) and safety aspects are often prohibitive. This paper describes a novel biological process for safe and cost effective production of sulfide from elemental sulfur, waste sulfuric acid or sulphate present in effluents. With this technology, gaseous or dissolved H2S is produced on-site and on-demand in an engineered, high rate bioreactor Experience with industrial applications at metal processing plants will be presented. The technology can serve to selectively recover metals from e.g. bleed streams, leach liquor, effluent streams and acid mine drainage. Lower overall costs and increased safety (no transport or storage of sulfide, production on-demand and at ambient pressure) are the main advantages of this new process compared to its alternatives.

Gerald J. Zagury et al. (2006) found that acid mine drainage (AMD), which contains high concentrations of sulfate and dissolved metals, is a serious environmental problem. It can be treated in situ by sulfate reducing bacteria (SRB), but effectiveness of the treatment process depends on the organic substrate chosen to supply the bacteria's carbon source. Six natural organic materials were characterized in order to investigate how well these promote sulfate reduction and metal precipitation by SRB. Maple wood chips, sphagnum peat moss, leaf compost, conifer compost, poultry manure and conifer

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sawdust were investigated in terms of their carbon (TOC, TIC, DOC) and nitrogen (TKN) content, as well as their easily available substances content (EAS). Single substrates, ethanol, a mixture of leaf compost (30% w/w), poultry manure (18% w/ w), and maple wood chips (2% w/w), and the same mixture spiked with formaldehyde were then tested in a 70-day batch experiment to evaluate their performance in sulfate reduction and metal removal from synthetic AMD. Metal removal efficiency in batch reactors was as high as 100% for Fe, 99% for Mn, 99% for Cd, 99% for Ni, and 94% for Zn depending on reactive mixtures. Early metal removal (0–12 d) was attributed to the precipitation of (oxy) hydroxides and carbonate minerals. The lowest metal and sulfate removal efficiency was found in the reactor containing poultry manure as the single carbon source despite its high DOC and EAS content. The mixture of organic materials was most effective in promoting sulfate reduction, followed by ethanol and maple wood chips, and single natural organic substrates generally showed low reactivity. Formaldehyde (0.015% (w/v)) provided only temporary bacterial inhibition. Although characterization of substrates on an individual basis provided insight on their chemical make-up, it did not give a clear indication of their ability to promote sulfate reduction and metal removal.

The summary of the above literature is given in the Table 1.

Table 1. *Summary of literature*

S.N.	Method of Removal of heavy metal	Metal	pH	Concent-ration	% Removal	Data	Authors Name
1	Amidoxime-containing modified starch cation exchange method	Cu, Hg Pb Zn	5	100-200 mg/l	-----	Distribution coefficients	B.W. Zhang et al. (1993)
Remarks Modified starch could acquire wide application in purification procedures for wastewater and enrichment of trace heavy metals in natural waters prior to analysis.							
2	Ion exchange	Zn, Cd Cu, Pb	5	200ppm	-----	Distribution coefficients	Yi-Min Gao Et al. (1995)
Remarks HISORB, a hybrid inorganic sorbent, can effectively remove low concentrations (Mg/l to rag/l) of dissolved heavy metals in fixed-bed processes with an influent pH as low as 3.5 from the background of much higher concentrations of competing calcium and sodium ions.							

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S.N.	Method of Removal of heavy metal	Metal	pH	Concent-ration	% Removal	Data	Authors Name
3	Biosorption and ion Exchange	Au Cd Co Cr Cu Hg Ni Pb Se Zn	< 6	10-100 ppm metal solution	>85 %	The range of metals bound, the effects of pH, temperature, contact time, interference by common salts and the effect of multiple cycles of metal binding and elution.	Jack Z. Xie, (1996)
Remarks Biosorbents and chemically modified biosorbents were prepared and compared with commercial ion-exchange resins to determine their metal-binding capacity.							
4	Fluidized bed reactor;	Fe	2	500 mg/1	90 %	--	Teruyuki Umita (1996)
Remarks Hydraulic retention time can be decreased by using the fluidized bed reactor.							
5	Algal Biomass Adsorption	Zn, Cu, Cd	2 - 8	500 mg/lit to 3000 mg/lit	60 to 85%	Effect of pH, Time Concentration	P.D. Rose (1998)
Remarks Utility of the waste stabilization pounding process, providing an established reactor technology for the treatment of large water volumes, and the feasibility of linking co-disposal of organic waste streams with AMD treatment.							
6	Anaerobic reactor with mixture of rice stalk, cow manure and limestone	Cd, Pb, As, Zn, Cu, Fe, Al and Mn.	6	70-126 mg/lit	98 to 100 %	Effect of Time	Young-Wook Cheong (1998)
Remarks The reactor was successful in raising the pH and removing metals to over 95%, except for Mn. Maximum removal efficiencies of Cu, Zn, Cd, Pb, Mn and Fe were achieved when pH and Eh values were respectively 6							

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S.N.	Method of Removal of heavy metal	Metal	pH	Concent-ration	% Removal	Data	Authors Name
7	Bioremediation	Sulphate	3-6	--	14.4 to 26.7 %	Effect of Time, pH	Phillip Elliott (1998)
Remarks Sulfate-reducing bacteria (SRB) were capable of sulfate reduction and production of alkalinity at pH values as low as 3.25.							
8	Continuously-stirred tank reactor	Fe	3.56 – 31.7	100-250 mg/lit	**--**	Field determination	Carl S. Kirby (1998)
Remarks A field continuously-stirred tank reactor has been successfully used to determine Fe2+ oxidation rates in acid and alkaline mine drainage.							
9	Microbial activity	Fe	4-5	300 to 92 mg/l,	32 %	Field determination	T. K. Tsukamoto (1999)
Remarks Results indicate that methanol and lactate can be used effectively as reducing equivalents for restoring the activity of sulfate-reducing microorganisms in a laboratory anaerobic bioreactor containing spent substrate.							
10	'Anoxic' limestone drains (ALDs).	Al Fe	3.5-7	34.3 mg/l	**---**	Dissolved oxygen (DO), pH values, and water table elevation Measurement at different depths	E.I. Robbins (1999)
Remarks The very large variability of microbial populations on the homogenous substances tested in this research suggest that reactions with heterogeneous alkalinity-producing materials such as CCBs and FGDBs will be difficult to predict in advance of performing the actual field tests.							
11	Precipitation.	Fe Mn	1.65 to 12.5	942 mg/lit 113 mg/lit	80-90 %	Sorption Isotherm	D. Feng, (2000)
Remarks Based on experimental results, the oxidation and precipitation of heavy metals with lime and subsequent sulfide-carrier magnetic separation appear to be particularly suitable for the removal of heavy metal ions from the effluent of the particular gold mine.							

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S.N.	Method of Removal of heavy metal	Metal	pH	Concent-ration	% Removal	Data	Authors Name
12	Biosorption	Zn Cu	3	700 mg/lit	74 mg Uptake	Langmuir Isotherm	Vivek Utgikar (2000)
Remarks This study was confined to synthetic solutions of Cu(II) and/or Zn(II), closely corresponding to typical AMD compositions, and equilibrium uptake of the metals was observed at pHs of 3.0 and 3.8.							
13	Organic waste material	Cu, Zn	6.8	50 ppm 100 ppm	85-90%	Effect of Time	In Seop Chang (2000)
Remarks Various waste materials could support bacterial sulfate reduction in packed bed reactors to treat acid mine drainage (AMD). A short-term experiment showed that raw biomass (OC) was less satisfactory for the purpose than biologically (SMC, SOS) or chemically (SWP) treated biomass.							
14	Chemical precipitation	Fe Mn	4.5	194 ppm 4.65 ppm	80 %	Leaching results	Matthew M. Matlock, (2002)
Remarks Current treatment processes for AMD, such as precipitation through neutralization and peroxide addition, have major disadvantages, including large doses of lime and peroxide and costly sludge disposal fees. The ability of BDET to reduce the concentration of the metal contaminates from the acidic waters directly from the mine emphasizes the compound's potential use as a treatment reagent. Due to the exceptional stability of the precipitates under oxidative and pH conditions, BDET can be utilized as an additive to reduce metal concentrations being discharged.							
15	Up flow anaerobic reactor	Fe, Al, Cu, Cd	6.3 – 6.9	279 mg/lit 13 mg/lit 68 mg/lit 56 mg/lit	99%	Effect of Time	Hyun-Joon La (2003)
Remarks The concentration of heavy metals in the effluent from the up flow anaerobic reactors was effectively removed.							
16	Electrochemical treatment	Fe Cu	6-8	64 ppm 58 ppm	80-90%	Proton removal efficiencies	M.M.G. Chartrand (2003)
Remarks Electrolysis of authentic AMD was successful in removing iron from solution, Development of an electrolytic technology for AMD remediation requires more work on the chronology of electrolysis, aeration, and sludge separation, and on cell design to optimize mass transfer and permit the in situ separation of the sludges formed when the original AMD contains significant quantities of Fe (III).							

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S.N.	Method of Removal of heavy metal	Metal	pH	Concent-ration	% Removal	Data	Authors Name
17	Anaerobic treatment;	Fe	3.5	100 mg/lit	93 %	Effect of Time	T.K. Tsukamoto (2004)
Remarks Ethanol and methanol were both successfully utilized to remove sulfate andiron in SRB column studies, although ethanol offers the advantage of a shortened acclimation time.							
18	Adsorption	Cu, Zn Mn	--	100 to 400 mg/l.	---	Langmuir, Freundlich, and Dubinin–Kaganer–Radushkevich (DKR) adsorption isotherm data,	E. Erdem (2004)
Remarks These results show that natural zeolite can be used effectively for the removal of metal cations from wastewater. This naturally occurring material provides a substitute for the use of activated carbon as adsorbent due to its availability and its low cost.							
19	Bioremediation	Fe, Zn, Cu	2.3 - 6	497 mg/lit 107 mg/lit 49 mg/lit	98 %	Time study	M. C. Costa (2005)
Remarks Further studies need to be carried out to control and maintain a stable sulfate removal for longer periods at values suitable for discharge on nature or for irrigation purposes.							
20	Passive Treatment	Fe Al Ni	6.5	Fe 2500 ppm Al 10.5 ppm Ni 15 ppm	Fe 99.95 % Al 100 % Ni 85.7 %	Geo chemical Modeling	Louis R. Bernier (2005)
Remarks The use of serpentinite to generate alkalinity in passive treatment systems design appears promising but more research, economic study and pilot testing is mandatory before full-scale implementation can be achieved. It is anticipated that perhaps in the near future, passive treatment using serpentinite will be implemented in the decontamination of mine effluents in areas where livestock drinking water is a rare resource and also contribute to the revalorization of asbestos (chrysotile) tailings if the economics are favorable.							

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S.N.	Method of Removal of heavy metal	Metal	pH	Concent-ration	% Removal	Data	Authors Name
21	Precipitation	Fe Al	3.5 – 4.0	Fe 172.5 mg/lit Al 88.6 mg/lit	Fe 98.6% Al 97.2 %	Time Study	Xinchao Wei, (2005)
Remarks Simultaneous metal recovery and AMD treatment were achieved using a selective precipitation process based on solubility characteristics of the major and minor metals in the AMD.							
22	Metal sulphide precipitation	Zn Cu Fe	4-6	Zn 660 mg/l Cu 19 mg/l Fe 337	Zn Fe	Installation of wastewater treatment plant	Jacco L. Huisman (2006)
Remarks The Thioteq technology is a new alternative for hydroxide precipitation based on safe and on-site hydrogen sulfide production. Biological reduction of elemental sulfur or sulfate produces low cost sulfide reagent that can be used effectively in water treatment for both environmental control and metal recovery. Commercial applications in industrial plants have shown the technology to be safe and robust.							
23	Passive biological treatment	Fe Mn Cd Ni Zn	3.9 – 4.2	Fe1683 mg/l Mn 15 mg/l Cd 11.3 mg/l Ni 16 mg/l Zn 18 mg/l	Fe 100% Mn 99% Cd 99% Ni 99% , Zn 94%	Geo chemical modeling	Gerald J. Zagury (2006)
Remarks The natural organic substrate mixtures (with and without formaldehyde) were successful in promoting sulfate reduction and metal removal (94–99%) but the lowest metal removal efficiency was observed in the reactor containing poultry manure as a single carbon source.							

Conclusion

Worldwide metal contamination of soil and water has severe impacts on environment and human health. Acid mine drainage is one of the most important sources of heavy metal environmental pollution. This review paper shows that the different removal techniques for heavy metal removal from acid mine drainage wastewater.

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METHANE EMISSION FROM TWO DIFFERENT RICE ECOSYSTEMS (*AHU* AND *SALI*) AT LOWER BRAHMAPUTRA VALLEY ZONE OF NORTH EAST INDIA

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(recevied 27th August 2007 ; accepted 21st July 2008)

Abstract. Monocropping of rice is practiced in Assam (situated at north east part of India) throughout the year in different agro-ecosystems (upland and lowland) primarily under rainfed conditions. The estimation of methane emission has been realized investigating high yielding rice varieties viz. Ranjit and Mahsuri, grown under two different agro-ecosystems at Lower Brahmaputra Valley Zone of Assam with sandy to sandy loam type of soil. Variety 'Ranjit' grown at monsoon (*Sali*) rice ecosystem at lowland rainfed condition showed higher seasonal integrated methane flux value compared to variety 'Mahsuri' grown at pre monsoon (*Ahu*) rice ecosystem. Both varieties showed two methane peaks, one at the active tillering stage and the second at the reproductive stage of the crop. The observed variation in methane emission peaks are contributed mainly by physiological characteristics of the rice plant such as leaf numbers, tiller numbers, plant height, root shoot biomass and leaf area index. Statistical analysis of these parameters showed a positive correlation with methane emission. These physiological parameters are in turn governed by plant genotypes and environment i.e., the field water availability and climatological factors (rainfall and temperature) during the growing season. While comparing the two ecosystems it was found that methane emission is significantly less from upland rainfed rice ecosystem and this ecosystem can be considered a suitable option for biological mitigation of methane from rice paddies.

Keywords: *Assam; India; rainfed ecosystem; water regime; growth parameters*

Introduction

Methane is an important greenhouse gas with strong infrared absorption bands trapping a large part of thermal radiation from Earth's surface. The increase of methane concentration in the global troposphere is currently around 9.9 ppb (v/v) y^{-1} of which nearly 60% is attributed by human activities [4]. Wetland rice fields are one of the largest agricultural sources of atmospheric methane. The increasing area of rice paddy fields in the world is considered to be an important cause of the recent shifts in the atmospheric methane balance.

The estimated methane budget from Indian paddy fields is of special significance as India has an area of about 42.2 M ha under rice cultivation, compared to China's 33.7 M ha. Several studies from India indicated that intermittent flooding could remarkably reduce the seasonal methane emissions (22-88%) without any loss in rice yield [22]. Rice plant characteristics have a strong impact on methane emissions and a negligible to substantial differences (up to 56%) in the rate between different cultivars were recorded [33]. The rate of methane emissions varies greatly among rice ecosystems because of the differences in water regime and in biological processes [24]. The agriculture land in

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Assam is predominately covered by rice which at present is grown mainly during the monsoon (July-Oct.) season. Monsoon rice (*Sali*) occupies 1.75 M ha, premonsoon (*Ahu*) rice occupies 0.524 M ha and the summer rice comprising of *Boro* occupies an area of 0.35M ha. The rice production area is increasing in Assam as in India, generally. This factor may lead to further increase in methane emission. Thus, an experiment was undertaken to determine the methane emission rate from rainfed (*Sali*/monsoon and *Ahu*/ premonsoon) rice fields of Lower Brahmaputra Valley Zone of Assam and to evaluate the soil and plant factors associated with emission of methane.

Review of literature

Methane, the major component of natural gas is the second most important greenhouse gas [28] with a current ambient concentration of 1.77 ppm [12]. Rice paddies, as an important anthropogenic source of CH_4, may account for about 15-20% of the global atmospheric methane budget. There is strong consensus among atmospheric scientists that global warming and climate change is the result of the accumulation of greenhouse gases in the global atmosphere [5, 34]. The average lifetime of each methane molecule that reaches the atmosphere is about 8-10 years [14] and its global warming potential in relation to CO_2 is about 26.9 for a ten-year integration period [16]. The first field measurement of CH_4 emission from rice paddy fields was conducted in California [7], followed by extensive studies in Spain, Italy, China, Indonesia, Japan, Australia, Korea and Philippines by various workers. In India, the CH_4 emission estimation was started from 1991 onwards [23]. Furthermore, a region specific characterization of agronomic (fertilizer application, water management, etc.) and natural factors (soil properties, weather patterns, etc.) is indispensable to cope with the extreme temporal and spatial variability in CH_4 emission from same rice cultivation systems [21] or different rice ecologies [33]. To meet the demand of the exploding human population, the world's annual rice production must increase from the present 520 million tons to at least 880 million tons by 2025 as rice is a staple food for more than half of the world's population [15]. With the intensification of rice cultivation by the adoption of modern agronomic practices, CH_4 emission from rice paddies is expected to increase [20]. Soils have important influences on the production, oxidation, accumulation of, and emission of CH_4.

The largest area under rice cultivation accounting about 28% of total crop area (42.3 M ha including the double crop areas) is in India [31]. Here, rice is grown at an altitude from below the sea levels to 2000 m above the sea level and from upland with no standing water for most of the growing period to deepwater (> 1m water depth as in many places in eastern India due to excess rainfall). Based on the depth of water stagnation in the fields, the rain fed lowland rice area in India is classified (Sharma *et al.*1995) into (i) Shallow lowlands (0-30 cm), (ii) Intermediate lowlands (0-50cm) (iii) Semi deep lands (0-100 cm) and (iv) Deep water lands (more than 100cm). But little information is available on the contribution of Indian rice fields to the global methane budget. So, in the present study attempts have been made to find out the contribution of different Indian rice ecosystem to global methane budget.

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Materials and methods

Field procedures

The experiment was undertaken during *Ahu/*monsoon (Feb-June) and *Sali/*premonsoon (June-Nov) rice growing season at Kahikuchi, under Lower Brahmaputra Valley Zone (LBVZ) of Assam. The LBVZ of Assam lies between 25°30′N and 26°54′N latitude and 89°40′E and 92°10′E longitude. Geographically, this zone is bounded by folded ranges of Himalayas in the north and Shillong plateau in the south. The mighty Brahmaputra river flows through the zone. The northern part of the zone is characterized by small hillocks and some low-lying areas here and there. The flood plains of the Brahmaputra, extending up to the river Jinjiram bordering Meghalaya province constitute the southern part of this zone. Soils of this zone consist of new alluvium on both the banks of the Brahmaputra and old alluvium towards the foothills. Soils are mostly sandy and sandy loam in texture. Soils are rich in organic matter (0.78%) and consist of both acidic and near neutral soils. The climate in the zone is humid subtropical characterized by a warm humid climate having hot summer. The average rainfall during the monsoon season (June-September) ranges from 1065 mm to 2498 mm followed by post-monsoon period (October-November) which receives an average rainfall from 11.1 mm to 250 mm.

The first experiment on methane emission was conducted during the *Ahu/*premonsoon rice growing season under rainfed situation. The high yielding rice variety Mahsuri was transplanted in a well-prepared paddy field of total area 630 m^2. The field was divided into 10 equal sized subplots (9 m × 7 m) and thirty days old rice seedlings were transplanted in the field at a spacing of (20 cm × 15 cm).The second experiment was conducted during the *Sali/*monsoon rice growing season. The high yielding rice variety Ranjit was transplanted in the field of total size 630 m^2 which was divided into 10 equal sized subplots (9 m × 7 m) and seedlings were transplanted with the spacing of 20 cm × 15 cm.

In both the experiments, fertilizers were applied at 40:20:20 kg N-P_2O_5-K_2O ha^{-1}. Half of the urea (20 kg ha^{-1}), full dose of P_2O_5 and K_2O was applied at the time of transplanting. Half of the remaining part of urea (10 kg ha^{-1}) was applied at tillering stage and other half (10 kg ha^{-1}) was applied at panicle initiation stage as per package of practices of Assam Agricultural University, Jorhat and also followed by the farmers of this region. Plant growth parameters (plant height, tiller number, leaf number, root biomass, shoot biomass) were determined at weekly interval. Leaf area was measured by using portable Laser Leaf Area Meter (model CI-203, CID USA). Soil samples were collected before transplanting and estimation of Fe, Cu, Mn and Zn was done by DTPA (diethlene-triamine penta-acetic acid) extractable method using atomic absorption spectrophotometer model AA-203, Chemito. Meteorological data of both the crop growing seasons (rainfall and daily temperature minimum and maximum) were recorded and are presented in *Fig. 1a* and *1b*.

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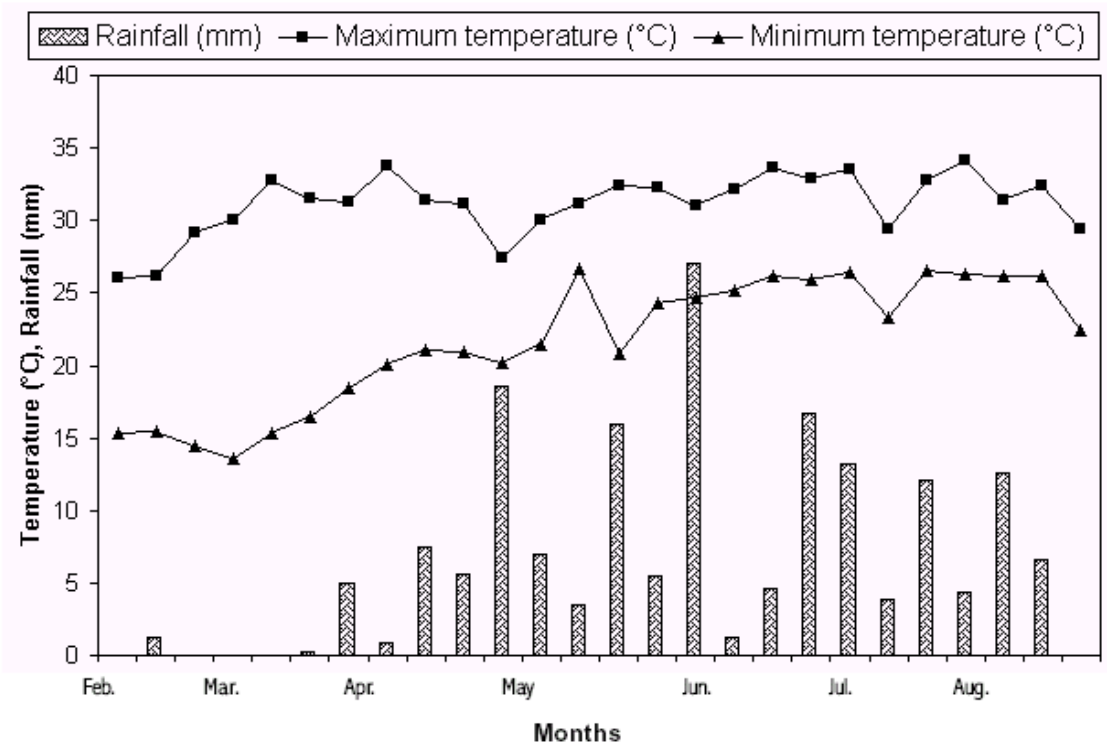

Figure 1a. *Meteorological data during premonsoon (Ahu) season of the experimental year at LBVZ, Kahikuchi, Assam*

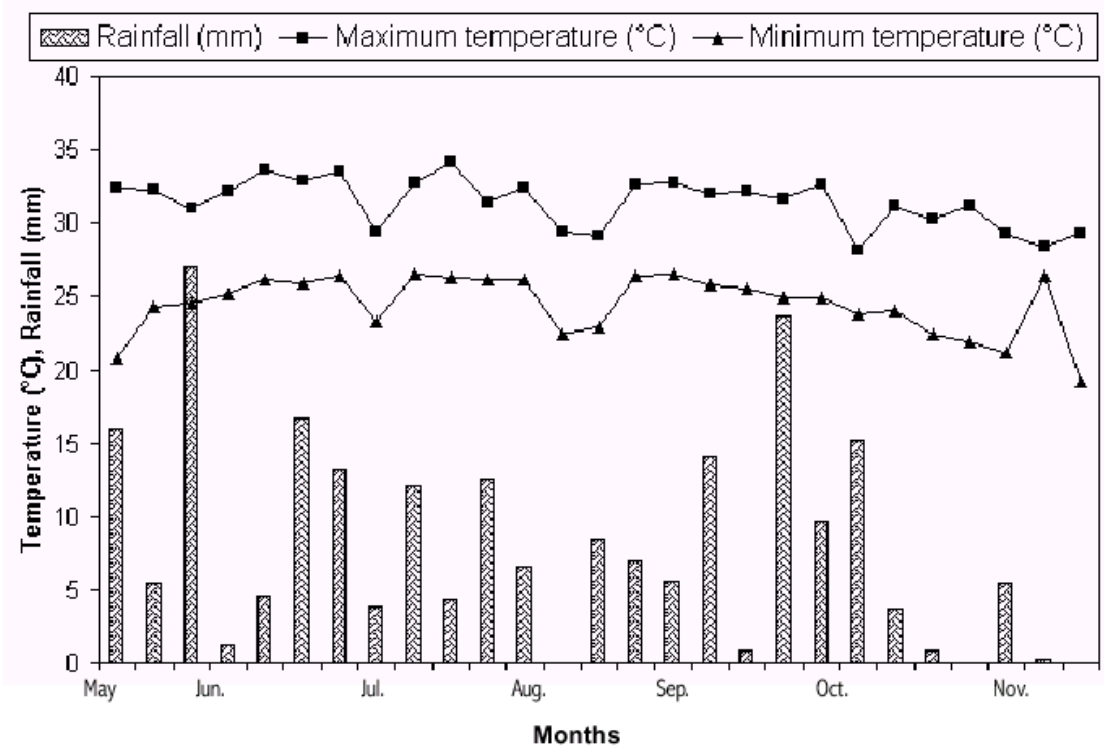

Figure 1b. *Meteorological data during monsoon (Sali) season of the experimental year at LBVZ, Kahikuchi, Assam*

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Methane emission measurement

The methane emission rate was measured by a static box technique [6]. At the measurement sites aluminium channel bases were fixed permanently, well in advance of sampling. The bases were mounted with a U-shaped channel to hold water. The Perspex box (30 cm × 50 cm × 70 cm) was then placed over the aluminium bases. The open end of the Perspex box rests on the channel. The water seal surrounding of the Perspex box made the system airtight. A battery-operated fan inside the Perspex box mixed the air in the chamber. The box was fitted with Tygon tubing terminating in a gas- tight stop cock and air samples were collected in glass bottles by a water displacement method. Glass air sampling bottles were fitted with three-way stop cock and a neck with self sealing silicon rubber septum. Samples were collected at fixed intervals of 0, 15, 30 and 45 minutes. Methane fluxes were determined once in a week in the morning starting from 14 days after transplanting and continued over the entire crop growing season.

After collection, samples were brought to the laboratory and concentration was determined by gas chromatograph (Varian model 3800) fitted with a flame ionization detector (FID) and Porapak N column (stainless steel column, 180 cm long and 0.32 cm**.** outside diameter). Column, detector and injector temperature were maintained at 90°C, 130°C and 130°C respectively. Nitrogen was used as the carrier gas, hydrogen as the fuel gas and zero air as the supporting gas. Ambient and box air temperatures, barometric pressure and water level inside the chamber were measured during each sample collection for calculating the chamber air volume at standard temperature and pressure (STP). Field water level, soil temperature, soil organic carbon content [13] and soil pH (Systronics Griph made 'D' pH meter 327) were determined during each methane sampling period. Methane flux (F) and seasonal integrated flux (E_{sif}) was calculated from the temporal increase in methane concentration inside the perspex box using the equation [27].

Statistical analysis was done to find out the correlation between methane flux and selected plant and soil parameters and the effect of selected parameters on methane emission was established.

Results

Methane emission during *ahu*/premonsoon season from rice cultivar Mahsuri grown in intermittently flooded situation under rainfed condition was recorded from 14 days after transplanting (DAT) of the crop. The first emission peak was observed at maximum tillering stage (56 DAT) and second peak was recorded at reproductive stage (91 DAT) (*Fig. 2*).The soil organic carbon during the crop growing period varied from 0.7% to 1.05% *(Fig. 3*). At the initial stage of crop growth fluctuations in soil organic carbon status were observed, with highest peak at 84 days after transplanting of the crop. Thereafter organic carbon of soil gradually decreased. Soil temperature increases slowly and reaches a maximum of 31°C just prior to panicle initiation stage (*Fig. 4a*). Soil pH ranges from 4.73 to 5.96 (*Fig. 4a*). Variations in field water level was observed depending upon rainfall and air temperature which decreases to a minimum level at initial stages (35 DAT and 49 DAT) and at pre-heading (84 DAT) stage of the crop (*Fig. 5*). The recorded soil moisture at these stages were 66%, 64% and 78% respectively. Leaf area index (LAI) is presented in *Fig. 6*. Shoot and root biomass of both the cultivars are presented in *Fig 7a* and *7b*.

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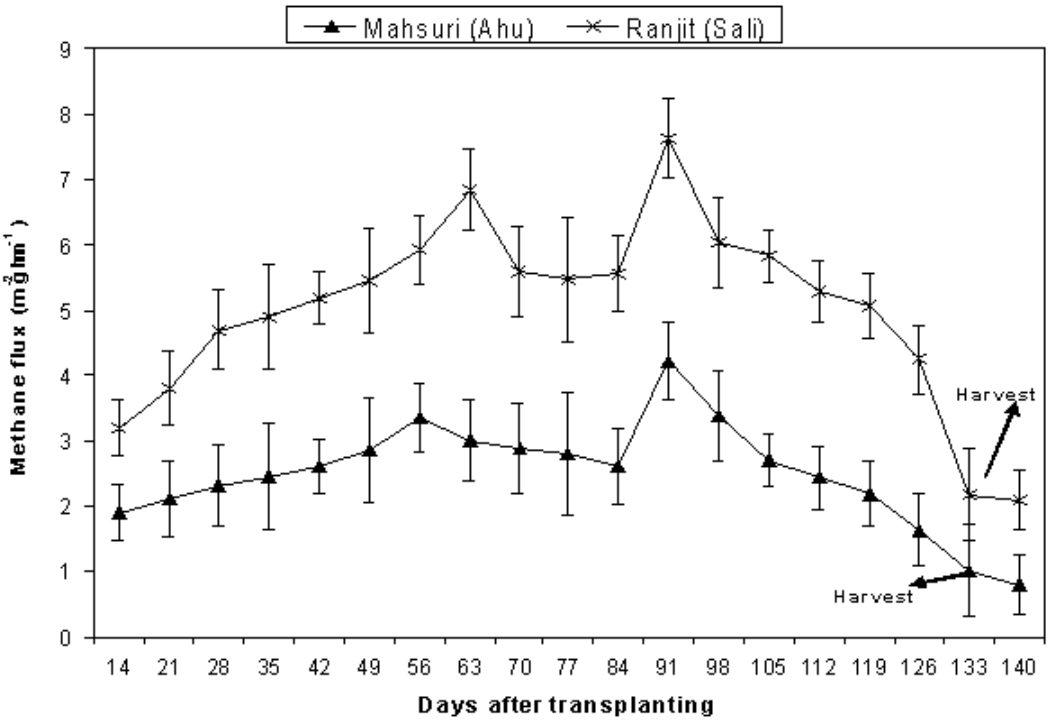

Figure 2. *Methane flux (mg m^{-2} hr^{-1}) from premonsoon (Ahu) and monsoon (Sali) rice growing season at LBVZ, Kahikuchi, Assam (Bars indicate ± S.E. values)*

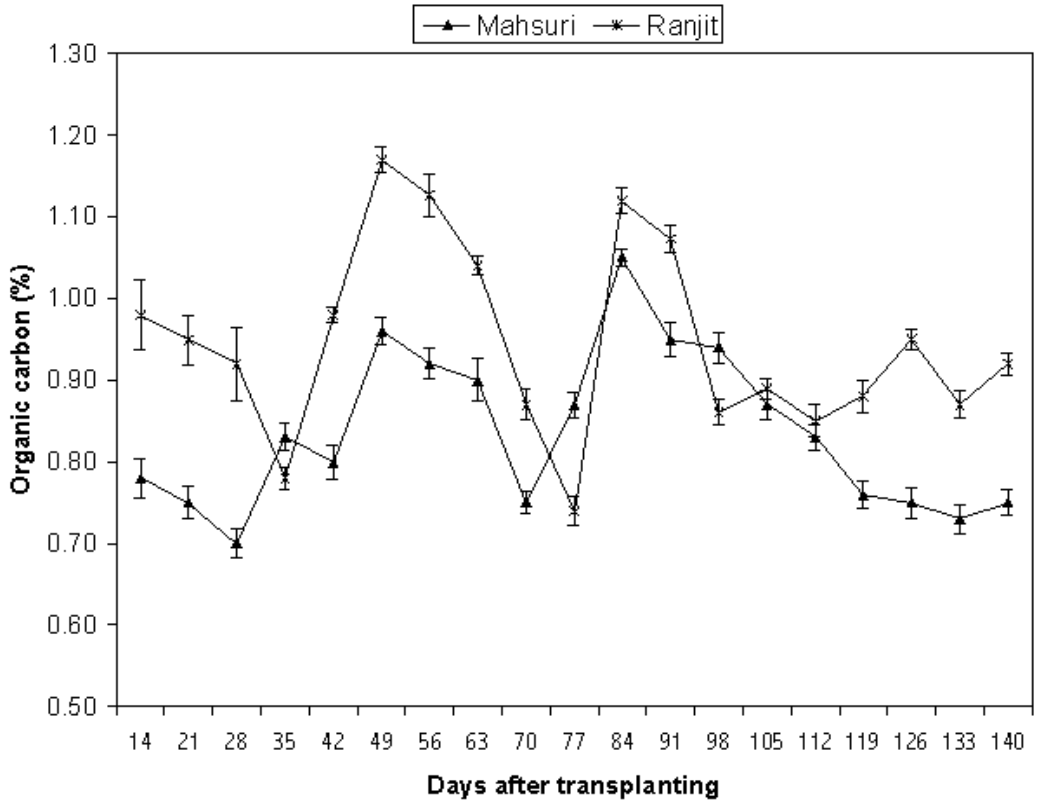

Figure 3. *Organic carbon (%) of the experimental field during premonsoon (Ahu) and monsoon (Sali) season of the LBVZ, Kahikuchi, Assam (Bars indicate ±S.E. values)*

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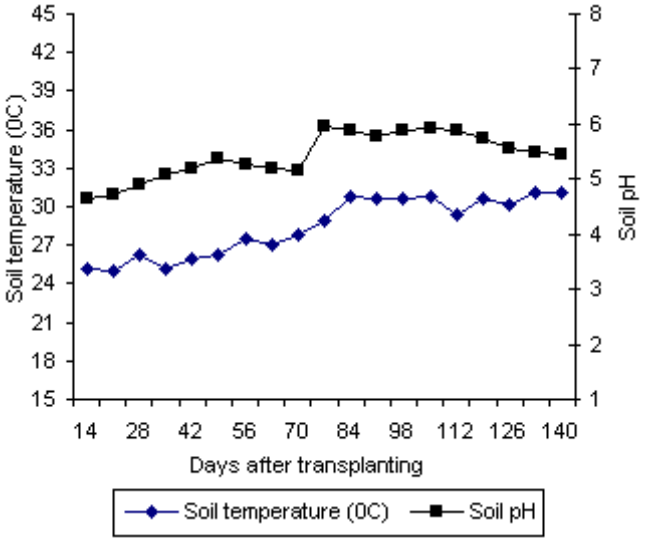

Figure 4a. *Soil temperature (°C) and pH of the experimental field during premonsoon (Ahu) season of the LBVZ, Kahikuchi, Assam*

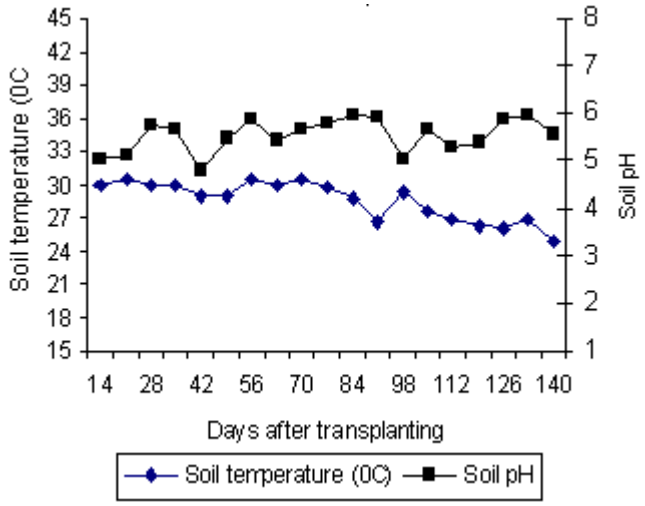

Figure 4b. *Soil temperature (°C) and pH of the experimental field during monsoon (Sali) season of the LBVZ, Kahikuchi, Assam*

Crop growth parameters such as plant height, leaf numbers, tiller numbers, root and shoot dry weight were recorded at weekly interval but the data presented in *Table 1 & 2* are of four different growth stages (maximum tillering stage, heading stage, 50% flowering stage and at harvest) of the crop. Methane emission over the entire crop growing season showed significant positive correlation with organic carbon status of the soil ($r = 0.557^{*}$) and leaf area index of the crop ($r = 0.485^{*}$) (*Table 3*).

Methane flux recorded under rainfed ecosystem from rice variety Ranjit during *Sali/*monsoon season at Lower Brahmaputra Valley Zone is shown in *Fig. 2*. Organic carbon content of soil reached a higher level at 49 and 84 days after transplanting of the

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crop *(Fig. 3*). The field remained continuously flooded till maturity *(Fig. 5).* Maximum soil temperature of 30.5°C was recorded at 70 DAT of the crop. Soil temperature gradually decreased towards maturity of the crop *(Fig. 4b).* Soil pH ranged from 4.79-5.99 *(Fig. 4b).* Leaf area index (LAI) trend was shown in *Fig. 6*. Data presented in *Table 2*. The variety Ranjit showed profuse vegetative growth as compared to variety Mahsuri in terms of leaf number, tiller number, plant height and root shoot biomass.

Table 1. *Paddy growth parameters during premonsoon (Ahu)season at LBVZ Kahikuchi, Assam*

Paddy variety/Parameters	**Growth stages**			
MAHSURI	**Active vegetative growth stage**	**Heading stage**	**50% flowering stage**	**At harvest**
Plant height (cm)	53.69	69.83	74.79	91.73
No. of tillers/hill	5.30	6.80	6.60	6.20
Leaf no./hill	21.10	29.20	30.10	10.50
Biomass(shoot g/hill)	2.54	8.20	9.99	11.32
Biomass (root g/hill)	1.73	3.27	3.38	2.53
Viable panicle/m^2				139.10
Panicle length (cm)				25.13
Sterility (%)				8.10
Yield (q/ha)				24.27
1000-grain weight (g)				18.09
Days to maximum tillering	56 DAT			
Days to heading		103 DAT		
Days to 50% flowering			109 DAT	
Days to harvesting				133 DAT

Table 2. *Paddy growth parameters during monsoon (Sali) season at LBVZ, Kahikuchi Assam*

Paddy variety/Parameters	**Growth stages**			
MAHSURI	**Active vegetative growth stage**	**Heading stage**	**50% flowering stage**	**At harvest**
Plant height (cm)	68.77	123.29	140.42	151.18
No. of tillers/hill	14.00	10.50	8.90	8.00
Leaf no./hill	56.40	42.50	29.90	18.20
Biomass(shoot g/hill)	7.50	22.72	27.90	38.72
Biomass (root g/hill)	3.95	10.07	10.90	10.79
Viable panicle/m^2				257.00
Panicle length (cm)				24.54
Sterility (%)				8.92
Yield (q/ha)				40.90
1000-grain weight (g)				19.73
Days to maximum tillering	63 DAT			
Days to heading		114 DAT		
Days to 50% flowering			122 DAT	
Days to harvesting				163 DAT

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Table 3. *Correlation between selected plant and soil characters and methane emission during premonsoon (Ahu) and monsoon (Sali) rice season at LBVZ,Kahikuchi, Assam*

Paddy varieties/ Parameters	Correlation (Methane)
Mahsuri	
Plant height (cm)	0.439
Tiller no./hill	0.507
Leaf no./hill	0.295
LAI	0.485*
Shoot biomass (g/hill)	0.648**
Root biomass (g/hill)	0.603**
Organic carbon (%)	0.557*
Ranjit	
Plant height (cm)	0.052
Tiller no./hill	0.592**
Leaf no./hill	0.664**
LAI	0.683*
Shoot biomass (g/hill)	0.268
Root biomass (g/hill)	0.195
Organic carbon (%)	0.248

*Correlation is significant at the 0.05 level
**Correlation is significant at the 0.01 level

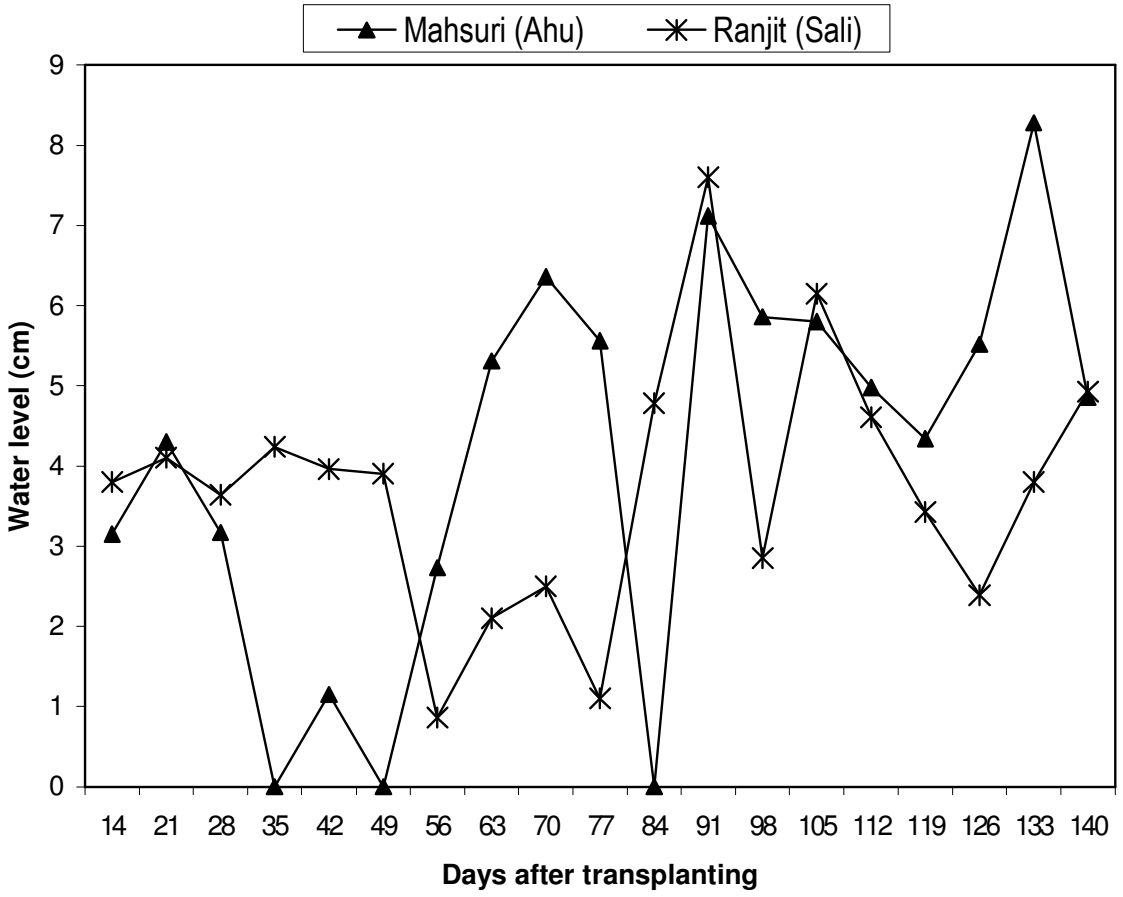

Figure 5. *Water level (cm) of the experimental field during premonsoon (Ahu) and monsoon (Sali) season of the LBVZ, Kahikuchi, Assam*

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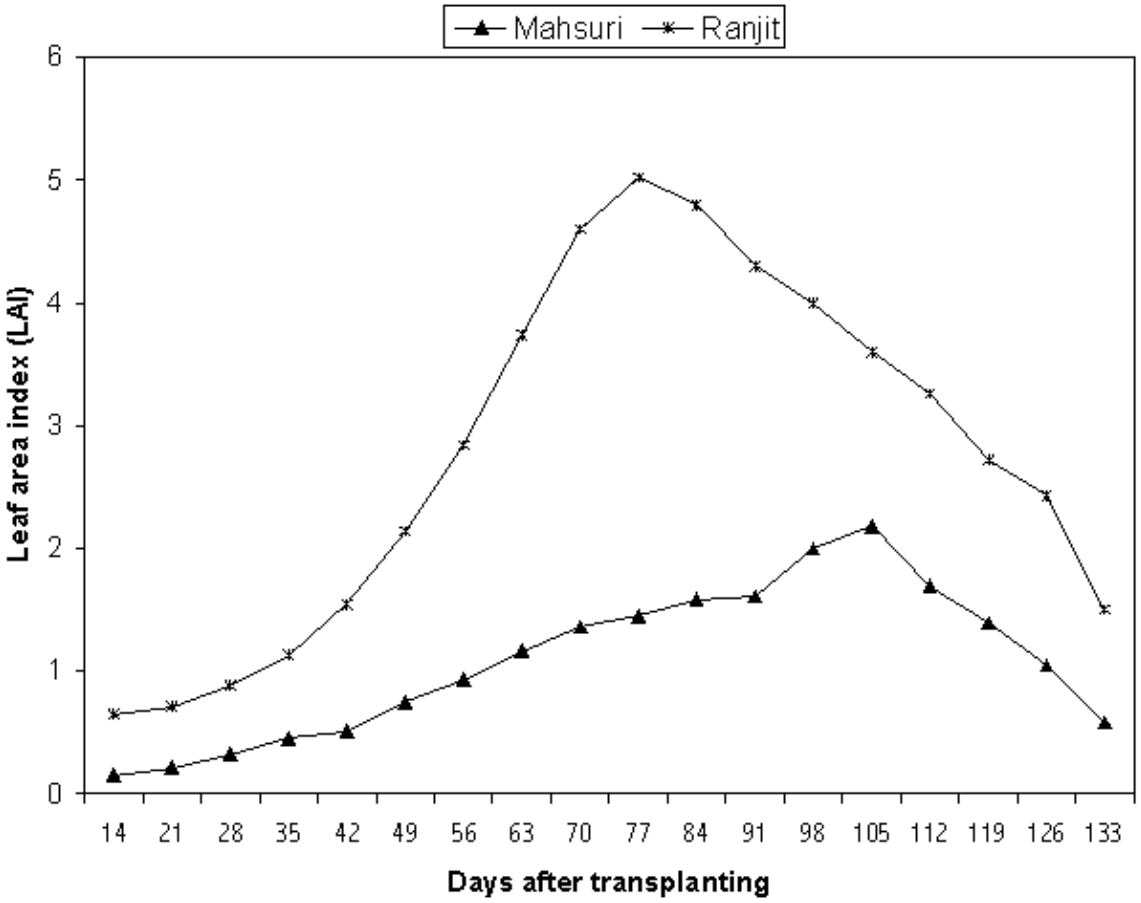

Figure 6. *Leaf area index (LAI) of the experimental crop during premonsoon (Ahu) and monsoon (Sali) season of the LBVZ, Kahikuchi, Assam*

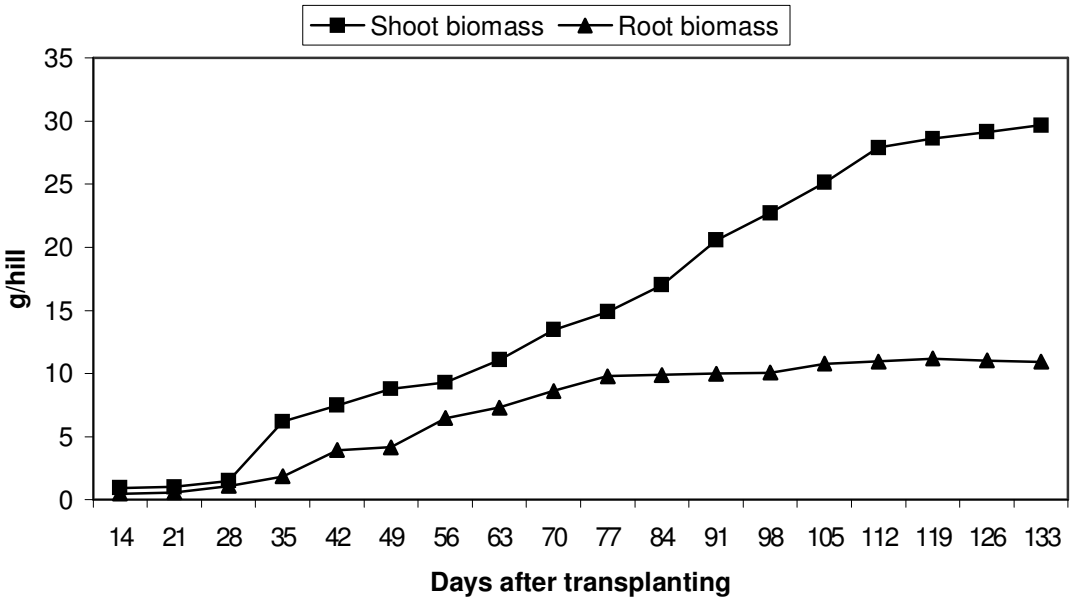

Figure 7a. *Shoot and root biomass of the experimental crop (cv.Ranjit) during monsoon (Sali) season at LBVZ, Kahikuchi, Assam*

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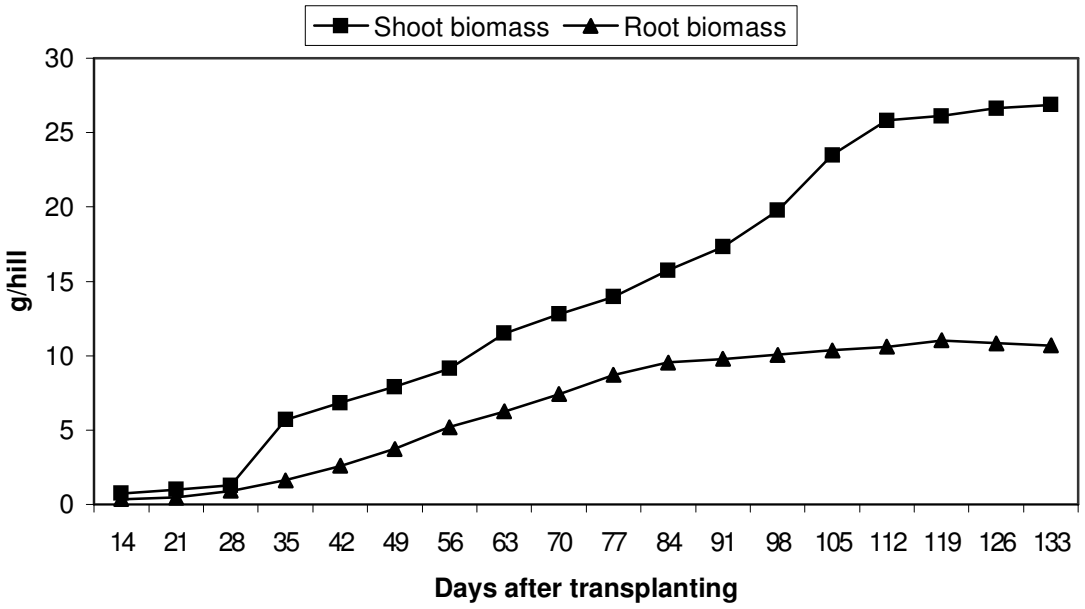

Figure7b. *Shoot and root biomass of the experimental crop (cv.Mahsuri) during premonsoon (Ahu) season at LBVZ, Kahikuchi, Assam*

Discussion

Lower rate of methane emission during early growth stages of rice plant is due to poor conduction of methane from the bulk reduced soil, to the atmosphere through the rice plants. The first peak at 56 days after transplanting, i.e. at maximum tillering stage of the crop, has been attributed to the decomposition of native organic matter in the soil which is left over as organic residues from the previous crop growing season thus increasing the substrate concentration for methanogenesis. It was reported that active vegetative growth stage of paddy at tillering enhances the transport of CH_4 produced in the rhizosphere through the rice plant to the atmosphere [3, 10, 11]. The second emission peak at 91 DAT (reproductive stage) is due to the additional organic matter from root exudates and root litters, which increases the carbon pool of the soil. It was previously reported that root exudates provide important carbon source for CH_4 production by supplying energy to the soil and also mobilize soil phosphorus and micronutrients [8].Increasing root system during rapid tillering increases the efficiency of gas transport through aerenchyma cells and intercellular gas space system by diffusion [2].

The cumulative methane emission did not show significant correlation with organic carbon status of the soil ($r = 0.248$). There is evidence that soil organic carbon alone may not be the major source for methane production. Other sources include organic carbon or the constant supply of carbohydrates and organic acids from the crop itself [9].Carbon as the substrate for methanogenic microorganisms may come from decayed organic matter, death of root tissues from the crop, and carbohydrate exudates from living root tissues. The second peak at 91 DAT might have been due to high availability of root exudates and root litters [8]. The decreased methane emission rate after panicle initiation stage in both the rice varieties could be due to reduced permeability of the root epidermal layer for ageing [26]. The experiments conducted in the same agroclimatic

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zone (Lower Brahmaputra Valley Zone) with similar soil physico-chemical properties, at two different agroecosystems showed variations in seasonal integrated methane flux. The E_{sif} value recorded during *Sali*/monsoon season was higher (16.39 gm $^{-2}$) as compared to E_{sif} value from *Ahu*/premonsoon season (7.51gm^{-2}).The difference in methane emission rate from two rice varieties grown under different agro-ecosystems were mainly due to higher shoot and root biomass accumulation in the variety Ranjit compared to variety Mahsuri (*Fig.7a &7b*). Plant biomass (both above ground and below ground) are known to have direct and indirect relationship with CH_4 efflux from rice fields [29].Larger root biomass provides more surface area for diffusion of CH_4 from adjacent reduced soil to the roots while larger above ground biomass in the form of stems signifies the conduit effect of rice plants [19].

An uniform soil temperature of 22°C to 27°C was recorded during monsoon/*Sali* rice compared to *Ahu* rice growing season (*Fig.4a* and *4b*). Most of the methanogenic bacteria are mesophilic with temperature optima of 30 to 40°C. Therefore, the higher temperature recorded during *Sali*/monsoon rice-growing season stimulate organic matter degradation which favors CH_4 production as well as limits accumulation of intermediate metabolites. Nodal development provides the major pathway of methane release to atmosphere [1], which may be the reason for higher methane emission in the variety with profuse vegetative growth.

It was observed that during premonsoon season (*Ahu* season) the rice field was completely dry representing oxidized situation, which is not congenial for CH_4 production and emission, is the reason for lower flux value recorded at this season. It has been established that water management in the rice field effect methane emission [3,30,36].Field drainage not only retards methane production but also promotes methane oxidation, produced in the preceding flooded regime, by methanotrophs. Thus in these experiments the intermittently flooded regime of premonsoon rice (*Ahu*) emitted less methane. Alterations of water regime may affect decomposition of organic matter in soil, which acts as substrate for methanogenesis [32].

This study revealed that rice cultivars grown under different agro-ecosystems in the same agroclimatic zone with identical soil physico-chemical properties show variations in methane emission rate. The rate of methane emission from a particular rice ecosystem is mainly determined by the water regime and other climatic factors (eg. Soil temperature, rainfall and availability of soil organic carbon). In the present study, the variety with profuse vegetative growth shows higher E_{sif} values. Based on the above facts rice varieties emitting less methane in a particular ecosystem with high yield potential may be considered suitable for cultivation. Further studies on screening of rice varieties are needed as suitable ecofriendly mitigation option.

Acknowledgement. We acknowledge the Department of Science and Technology Govt. of India for providing financial support for this work. Sincere thanks are due to Dr. N.K. Mohan, Chief Scientist, Regional Agricultural Research Station, Kahikuchi, Guwahati for his kind cooperation during the period of investigation.

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EXAMINATION OF WATER QUALITY FROM THE YARQON RIVER (CENTRAL ISRAEL) USING THE GLASS SLIDE METHOD TO DEFINE ALGAL VEGETATIVE ACTIVITY (*IN VITRO*)

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(Received 29th August 2007 ; accepted 15th March 2008)

Abstract. The provision of uniform, artificial substrates for periphytic colonization has a long history, and it is the most widespread of all the techniques applied to study algal periphyton activity. The Yarqon River is one of the largest coastal rivers in Israel. An experiment for the determination of algal vegetative activity in the artificial pools, using the glass method, from the Yarqon River stations was conducted during the period from 8.11.06 till 6.12.06. During the experiment we revealed the colonization's speed of substrates and the activity of self-purification processes at different stations of river. The artificial colonization of substrates by algae during the rainy period takes about 2-3 weeks. Chlorophyll, which was used as a trophic marker, revealed ultra-oligotrophic or oligotrophic levels in pool I. At pools II and III levels were either oligo-mesotrophic or eutrophic. These results corresponded to the gradient of organic matter in each experimental pool.The algal diversity found on glass slides represented about 10-15 species for each pool. During the experiment, cells from pool I were about 1-2.5 μm^3, and at pool Ii cells were about 0.1-1.4 μm^3. The increase of the ecosystem's entropy is indicative of environmental stress. This criterion, which is based on diversity dynamics, cell counts, and biomass may be used for monitoring the quality of water and aquatic ecosystems in the rivers of Israel.
Keywords: *glass side method, algae, diversity, density, artificial substrate, chlorophyll, ecology, Israel.*

Introduction

Nowadays, great attention is given to biological monitoring to assess the quality of aquatic ecosystems and to complete chemical and physical analyses. Aquatic organisms that integrate all biotic and abiotic parameters in their habitat can provide a continuous record of environmental quality and reveal various environmental changes of natural and anthropogenic origin. Benthic algae, diatoms in particular, possess many of the attributes required for monitoring organisms (McCormick, Cairns 1994; Lowe, Pan 1996): they are widely distributed and occupy an essential position at the base of aquatic food-webs as important primary producers in many freshwater environments; they are fixed to substrates, therefore integrating real conditions of the habitat, and respond more rapidly to environmental changes than higher level organisms because of their short life cycle; in addition, algal assemblages are species rich, composed of tens of taxa with various environmental tolerances and preferences. The provision of a uniform artificial substrate for periphytic colonization has a long history, and it is the most widespread of the techniques applied to studies of algal periphyton activity. One of the advantages of using artificial substrates is the possibility to determine the exact age of the algal community growing on them. If glass slides are used, it is not necessary to scrape off the algae from the substrate; the slides can be examined directly under the microscope (Gold et al. 2002). This method has many advantages: (a) not a single

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species is lost or destroyed, (b) interrelations between species can be studied more accurately, and (c) quantitative measurements are possible.

The Yarqon River is one of the largest coastal rivers in Israel. There is a clear distinction, based on water quality, between the upper and the central sections. While the upper section is relatively unperturbed with a low nutrient level, the central section is highly impacted by municipal effluents via the Qane tributary (Gafny et al. 2000). Tavassi et al. (2004, 2007) classified the level of water quality by evaluating the value of the Water Quality Index (WQI), basically a mathematical means of nine water quality parameters, along the Yarqon River. The upper section had a WQI value that represented good water quality, while water from the upper segment of the central section corresponded to fair water quality, which could be related to the confluence with the Qane tributary, where municipal effluents from a wastewater treatment plant entered the Yarqon River.

The quality of surface water is determined by nutrient loads penetrating the water basin, as well as the intensity in which self-purification processes occur. The algae serve as primary producers in the nutrients' utilization process; in rivers they develop on substrates. The intensity of the self-purification process is directly linked with the amount of nutrients and the speed of their utilization. Within the water monitoring framework in countries of the European Union, the rivers' periphyton is monitored (European Parliament 2000). For this purpose, the species composition in the algal assemblage, the presence of indicator species, abundance, species biomass in the community as well as chlorophyll concentrations are determined (Cardoso et al. 2005). Observation of substrates occupation is recommended during the vegetation period (Ács et al. 2005).

We know after several years of investigation of Israeli rivers that the algae are more abundant in the winter rainy season. In order to make any conclusions regarding the intensity of proccesses, it is essential to determine the attributes of four general classes: (1) taxonomic composition, (2) species richness and diversity, (3) tolerance/intolerance, and (4) trophic structure (King, Richardson 2003). An experiment for the determination of the algal vegetative activity in the streams of the Yarqon River was conducted during the period from 8.11.06 till 6.12.06. The purpose of this study was to reveal the speed of substrate colonization, the activity of the self-purification processes under various organic loads, and reveal trophic level by chlorophyll concentration, algal diversity and abundance (Table 1). This study was based on water collected from the Yarqon River in October 2006 from three different sites (Figure 1): pool I contained water collected from an upper section; pool II contained water from the upper section that was diluted with water from the Qane tributary at a ratio of 5:1; and pool III contained water collected next to the effluent of the Qane tributary of the Yarqon River.

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Table 1. *Trophic classes according to chlorophyll and algal cells number (Felfoldy, 1987)*

Trophic classes	Chlorophyll mg/m^3	Algal cells number $10^6/l$
atrophic	0	0
ultra-oligotrophic	< 1	<0.01
oligotrophic	1 – 3	0.01 - 0.05
oligo-mesotrophic	3 – 10	0.05 – 0.1
mesotrophic	10 – 20	0.1 – 0.5
eso-eutrophic	20 – 50	0.5 – 1
eutrophic	50 - 100	1 – 10
eu-polytrophic	100 - 200	10 – 100
polytrophic	200 – 800	100 – 500
hypertrophic	> 800	> 500

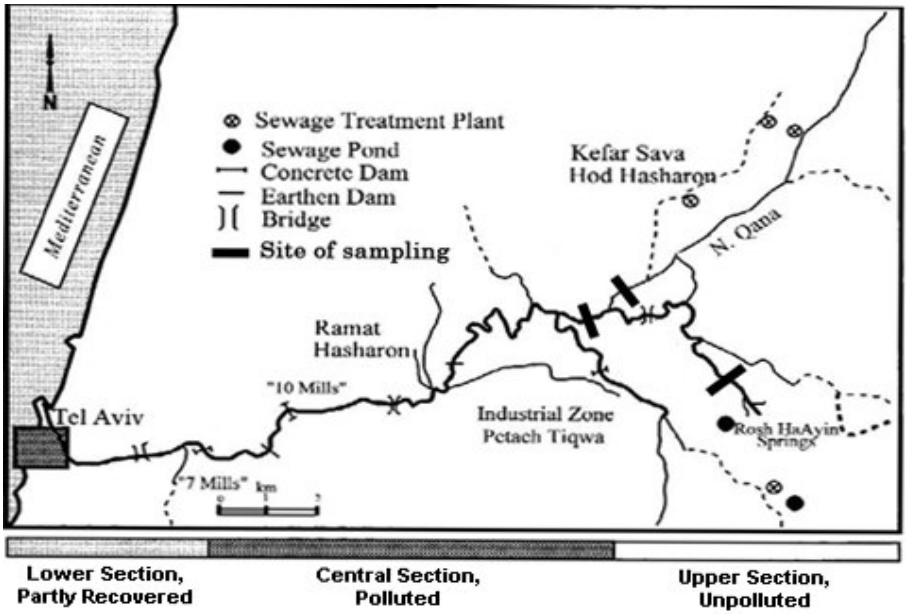

Figure 1. *Region of investigations: Sampling stations on the Yarqon River*

Materials and methods

The experiment was conducted *in vitro* on artificial substrates. Water samples (each 10 liters) as well as substrates and algae samples were collected from the habitats at the three sampling stations in the Yarqon River (Figure 1). For each sampling station the collected water, substrate, and algae where mixed together in a plastic container of 10 liters.

The pool-shaped containers were mixed and placed on the roof of one of the buildings at Haifa University. Daily measurement of temperature, conductivity,

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mineralization, and pH were performed using the HANNA HI 9813 apparatus and a thermometer. Glass slides served as the artificial substrates. They were placed horizontally in parallel to the water surface on buoys at a depth of 5 cm (Ács et al. 2005).

Measeurments of chlorophyll concentration on the glass slides and the nitrate concentration in the water were performed at 7-day intervals. The determination of chlorophyll concentration on glass slides was performed on the algal growth removed from a 10-cm^2 area on each slide. We were chosen methanol method because our algal communities was very diversed and enriched diatoms and cyanoprokaryotes (Thompson et. al. 1999). The chlorophyll concentration was determined in 3 repetitions using the spectrophotometric method for methanol extracts (Wetzel, Westlake 1969). In parallel to the chlorophyll concentration measurements were taken every The purpose of this study was to reveal the speed of substrate colonization, the activity of the self-purification processes under various organic loads, and reveal trophic level by chlorophyll concentration, algal diversity and abundance 7 days and the presence of algal species were determined: their abundance/number and biomass on each slide. The identification of algal species on the surface of the glass slides was performed using a dissecting Swift microscope under magnifications of 800. Cell counting for each species was performed via the direct counting method.

The existent methods for calculating periphyton algae on natural substrates are time consuming and complicated (Biggs 1988, 1996; Barinova, Medvedeva 2004; Sladečkova 1962). In our case, for artificial substrates, it was possible to simplify things, we used a widely known method (PhycoTech 2007), in which the accretions grow on a flat surface with known area. Counting of cells in each field of view (330x330 μm) was repeated 10-100 times for each species, and the average was registered. The final data appear in units of cells/cm^2. The calculation of the biomass was performed using the average volume of cells for each species. The average cell volume was determined by the measurement of numerous cells of the species and then looking at them using the known volume of a similar geometrical body (Hillebrand et al. 1999). The obtained cell volumes were multiplied by the previously obtained cell numbers.

Results and discussion

During the experiment period, water temperature range varied between 17-21^oC for the whole month. The pH values (Table 2) were consistently higher, along all the periods of the experiment, comparing pool III with pool II and pool I. The fluctuating changes in electric conductivity and mineralization (TDS) seem to have opposite trends (Figure 2), while for the first two weeks there was a moderate decreasing, which could be related to the self-purification process, for the following two weeks it sharply increased, which could be related to evaporation of the water from the artificial pools. However, at pool I the decreasing trend continued only for 8 days, meaning shorter self-purification process needs. In addition, it's reflecting the general differences between total dissolved solids in the water of upper section (pool I) and in the water of central section (pool III).

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Table 2. *The pH value in the artificial pool for 28 days.*

Pool	Min.	Max.	Ave.
I	6.5	7.2	6.8 ± 0.2
II	6.7	7.4	7.1 ± 0.2
III	7.0	7.7	7.4 ± 0.3

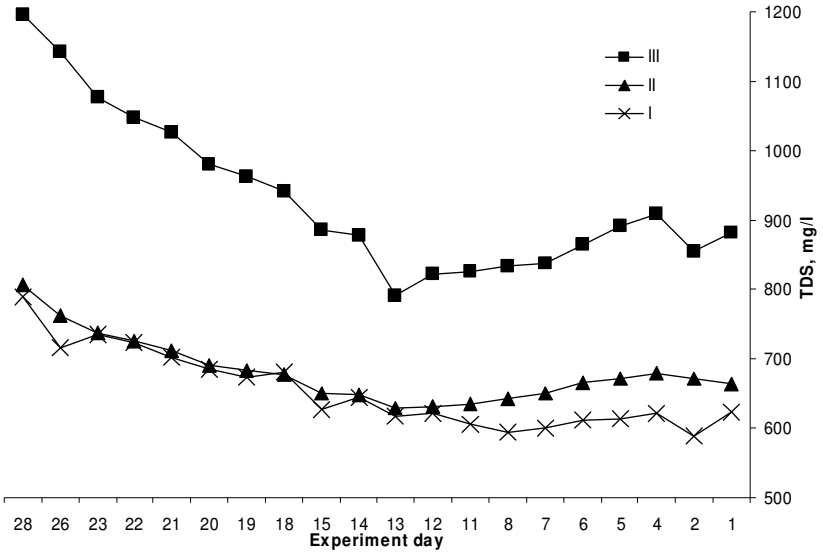

Figure 2. *Total Dissolved Solids (TDS) fluctuation in the experimental pools.*

The chlorophyll concentration represents the biomass of the periphyton community that attached on the glass slide. In rivers the periphyton abundance and composition correlates to the streaming speed (Bourassa, Cattaneo 1998) and the nutrients' concentration (Biggs 2000). Trophic status classification, based on chlorophyll concentration and cell number that was adopted for our data (Padisák et al. 1991; Dodds et al. 1998) for pool I where the chlorophyll concentration was very low during the whole experiment time, consistent with the utra-oligo- till oligo- level, while in pool II and III the concentration was much higher represent oligo-meso- till eutrophic level. The peak of the chlorophyll at pool II and III appeared in the second week, increasing by 2.2 and 2.8 in compare to the first week, while in pool I peak appeared at the third week (Figure 3). In pool III the chlorophyll concentration was much higher compared to the two other concentrations, i.e., in the second week, chlorophyll concentration was higher ~ 4.4 times than pool II, and ~ 68 times higher than pool I (Figure 3).

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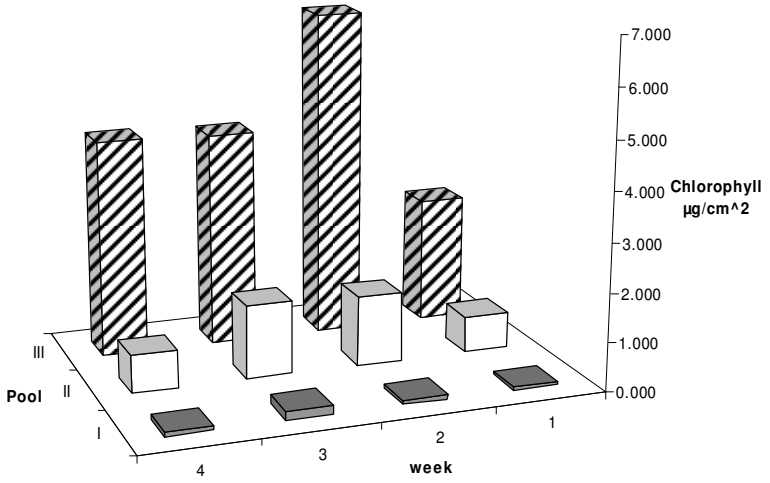

Figure 3. *Fluctuation of chlorophyll concentration in the experimental pools.*

Algal abundance in pool I reached a maximal level in the 3rd week of our experiment. The number of species kept growing even in the 4th week (Figure 4). These facts are indicative of a healthy ecosystem that allowed the development of algal community with high diversity. At pool II, despite algal diversity reaching a maximal level in the 3rd week, the number of cells kept growing with high rates (Figure 5). However, at pool III algal diversity and abundance changed almost simultaneously reaching a maximal level in the 2nd - 3rd week of our experiment (Figure 6), which may be related to the adaptation of the algal community to the high-organic load condition.

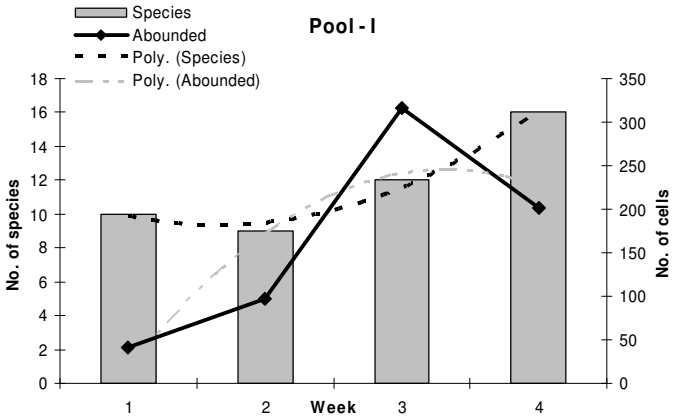

Figure 4. *Algal diversity and abundance fluctuation in the pool I.*

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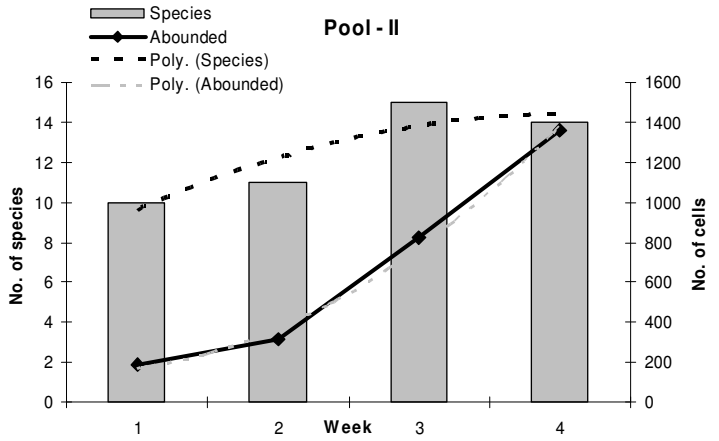

Figure 5. *Algal diversity and abundance fluctuation in pool II.*

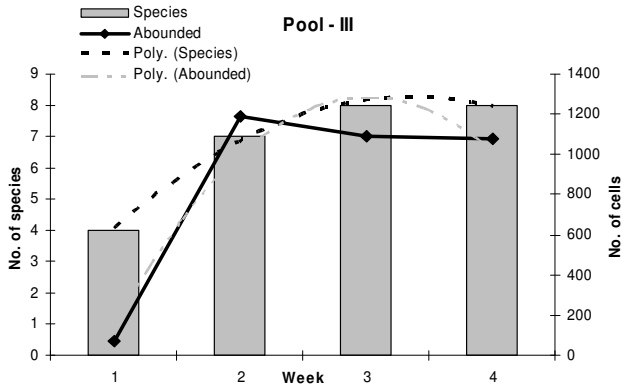

Figure 6*. Algal diversity and abundance fluctuation in pool III.*

From an analysis of taxonomic dynamics of the algal composition in the substrate colonization process, it can be seen that in pool I was maximal number of species and the maximal algal diversity with prevailing of Diatoms (Figure 7). This shows us that the experimental conditions were sufficient good for their development.

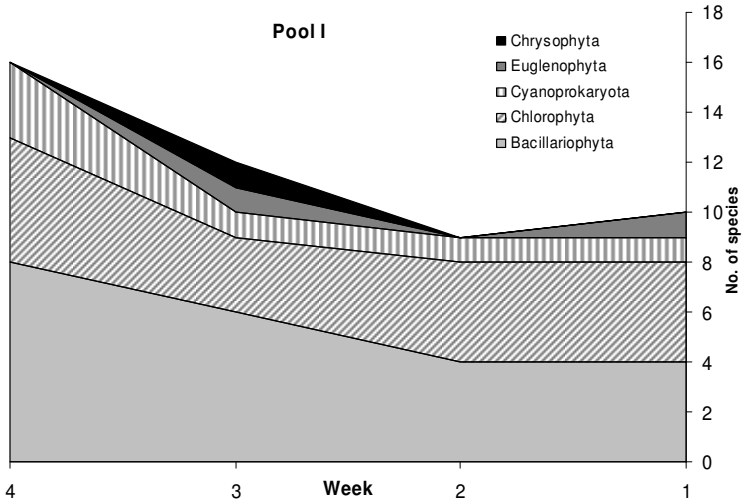

Figure 7*. Taxonomic dynamics of the algal composition in the substrate colonization process in pool I.*

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In pool II the maximal number of species and the maximal algal divisional variety were observed in 3 week, where the no. of green algae was increasing (Figure 8). This shows us that the mixed condition in the pool II were more enriched with nutrients.

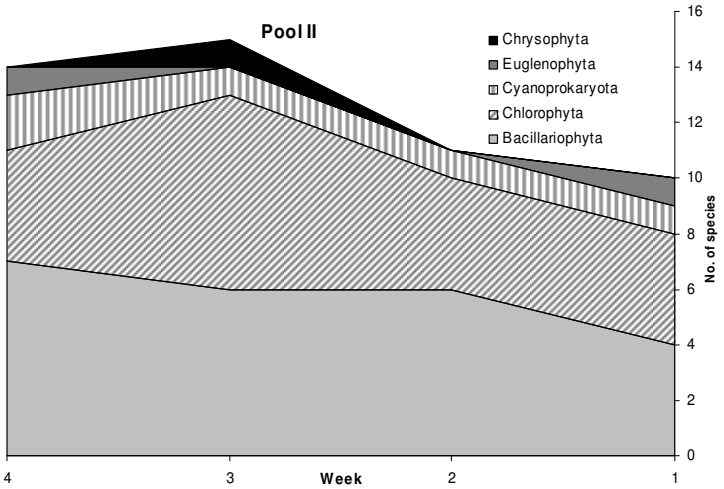

Figure 8. *Taxonomic dynamics of the algal composition in the substrate colonization process in pool II.*

However, in pool III we can see an increasing no. of species throughout the experiment. It's defined the higher number of euglenoid species and cyanoprokaryotes in compare to other taxonomic division (Figure 9). This increase could be related to the higher content of nutrients in the water from the Qane tributary.

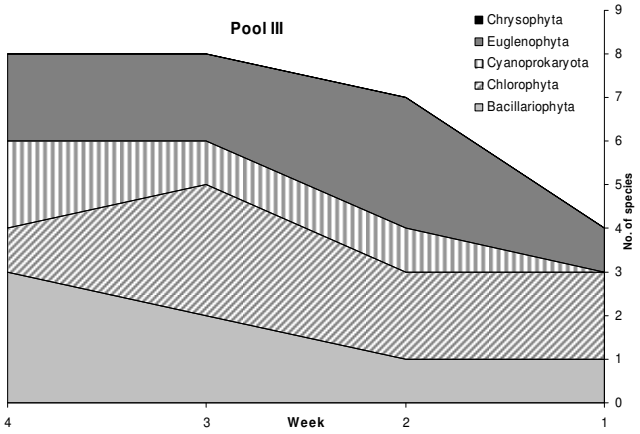

Figure 9. *Taxonomic dynamics of the algal composition in the substrate colonization process in pool III.*

The correlation between the number of cells and their total biomass reflects the condition of the ecosystem. Usually, in a case like this, the Shannon index is employed; however, we feel that the regularity can be better observed via the specific cell volume value.

In stable ecosystems the cells are bigger, and the growth rate isn't high. However, when the ecosystem is under the influence of stress factors (such as anthropogenic influence, usually caused with increasing of the trophic base) there is a pick in

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productivity, caused with increasing of total cells number and significant decreasing of cell volume.

In Figure 10, we can see in pool I simultaneous trends of algal development in respect to biomass and cell abundance with a maximum on 2 or 3 weeks. However, bio-volume of cells very rapidly decrease, and trend line of this process was opposite for biomass and abundance trends. During the third week cells number on substrate was much higher, and the bio-volume was lower than in the whole experiment time. This may be an indication of stress on the ecosystem that provides the Qane tributary.

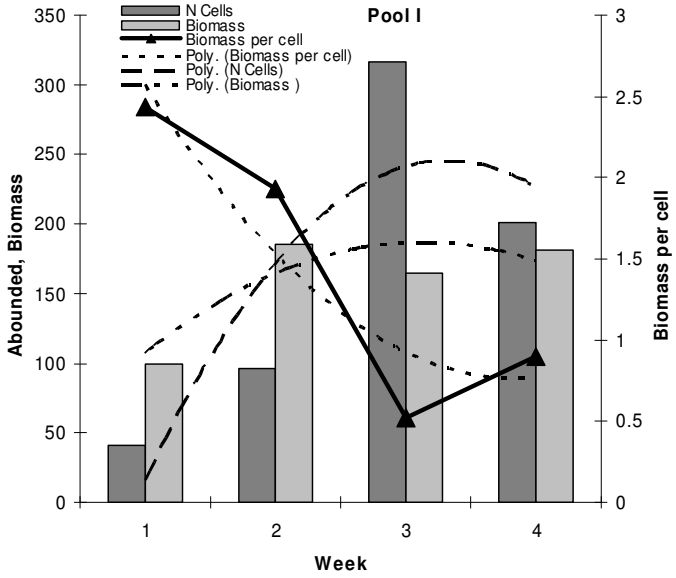

Figure 10. *Algal biomass and cell numbers fluctuation in pool I.*

In pool II biomass and abundance of algae were increased throughout the experiment, even in the last week (Figure 11). However, the bio-volume of cells was decreased in the second week. In comparison to pool I we see that the process of primary production was more rapid, and the ecosystem potential was not under nutrient limitation.

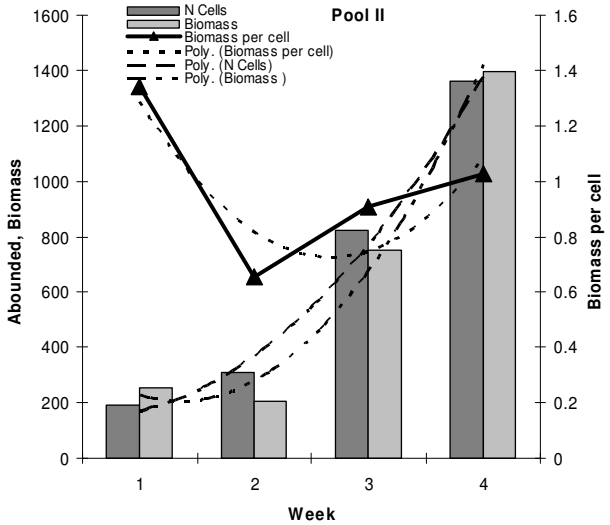

Figure 11. *Algal biomass and cell numbers fluctuation in pool II.*

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In pool III maximum algal biomass and abundance was revealed in the second week with a higher increase compared with the first week (Figure 12). These characteristics indicate a high level of organic load in the Qane tributary. However, a fivefold decrease of bio-volume of the cells from the first week to the third week shows toxic influence to the ecosystem health.

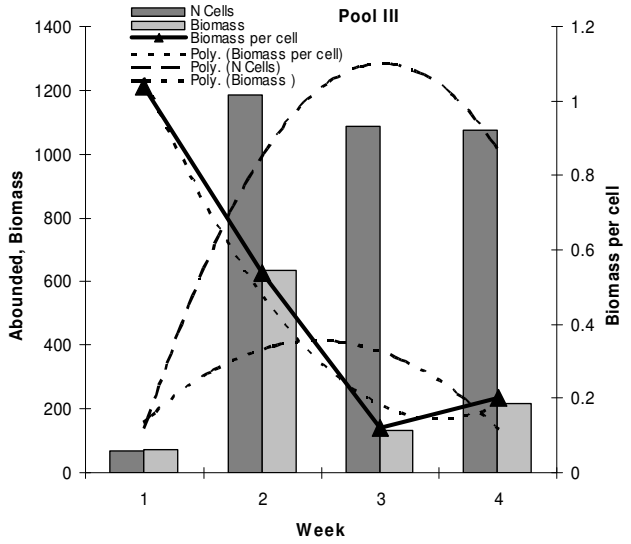

Figure 12. *Algal biomass and cell numbers fluctuation in pool III.*

Throughout, the experimental calculations show significantly decreasing of the bio-volume of cells after the second and third week. Therefore, our experiment was influenced by some level of stress.

It is a known fact that the higher the diversity, the more complex is its structure (Stirling, Wilsey 2001). However, if the trophic base grows in the early stages of the ecosystem's development, it leads to a steep rise in both diversity and biomass, followed by a plateau reached for all indices (Tilman 1996), exactly as observed in pool I.

In addition, in pools II and III there is a significant increase in the number of non-diatom algal species, mainly green and euglenoid algae, which indicates the presence of a large trophic base (larger than the one presented in pool one) (Heiskary, Markus 2003). In pool I the diversity reached its peak in the last week. The data reflect the potential possibility of the self-purification process even after four weeks that were followed by the lowering of the trophic level. In the pools that were enriched by nutrients (II-III), we can see a stabilization of species diversity after the second and third weeks.

In this manner when comparing the diversity and abundance charts (Figures 4-6, 10-12) it can be seen that the water from the Nofarim station (pool I) is relatively unperturbed with a lower nutrient level than the water from the two other stations. The temporal variability of ecosystem properties decreases with increasing diversity (Tilman 1996). Therefore, we see that the ecosystem in pools II and III is under the influence of a stress factor throughout all of our experiment.

A comparison of chlorophyll dynamics and cell abundance (Figure 3, 10-12) shows that after the passing peak in chlorophyll concentration at all experimental pools there is a decline in its concentration, accompanied by a decrease in cell size. This allows us to

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assume that the weak influence of stress factors continued during the whole experiment. However, these stress factors did not lead to the destruction of the ecosystem, since the biodiversity may be used as a buffer against environmental fluctuations (Thébault, Loreau 2005).

Conclusion

As can be seen from the above data and calculations, water temperature during the experimental period was typical for the autumn season (17-21^{o}C); the pH was previously alkali, and as a result of acidification was neutral; TDS decreased during the first two weeks, and afterwards increased as a result of evaporation. The prevailing evaporation influencing the ecosystem parameters at each of the stations confirms that the self-purification process came to an end. Chlorophyll, which was used as a trophic marker, had a low value at pool I, corresponding to an ultra-oligotrophic till oligotrophic level of the ecosystem. However, at pools II and III we revealed oligo-mesotrophic till eutrophic level of the ecosystem. These results revealed a gradient of organic matter in the experimental pools. This trophic level assessment is relevant to chemical analysis and indices of pollution, which we describe previously (Tavassi et al. 2005).

The algal diversity found on the glass slide accretions in each of the experimental pools was of about 10-15 species each. At the Nofarim station, maximal diversity was reached by the end of experiment, but, at the other stations, was already observed by the 3nd week. Algal cell counts in pools II and III were significantly higher than those in pool I.

During the experiment cells from pool I grew larger (1-2.5 μm^3); big cell species had some advantage in those systems, in comparison to the two other pools (0.1-1.4 μm^3). This process of cell size decreasing, which appears in all experiment pools, causing an increase in ecosystem entropy indicates that the ecosystems are under environmental stress.

Overall, the experimental study showed that colonization of artificial substrates by algae during the rainy period depends on an initial trophic level and takes about 2-3 weeks. A detailed observation of the self-purification process can be conducted by monitoring diversity dynamics, cell counts, and biomass. Mean specific cell surface or the mean specific biomass in the ecosystem can serve as “system health” criteria for water objects. These criteria may be used for the monitoring of water quality for aquatic ecosystems and in the management of rivers, wastewater reservoirs, etc., in Israel.

Acknowledgements. We thank Yonatan Raz from the Yarqon River Authority, who collaborated on chemical analyses. This work has been partially funded by the Israel Ministry of Absorption.

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ORIBATID ASSEMBLIES OF TROPICAL HIGH MOUNTAINS ON SOME POINTS OF THE „GONDWANA-BRIDGE" – A CASE STUDY

(METHODOLOGICAL POSSIBILITIES OF COENOLOGICAL INDICATION BASED ON ORIBATID MITES №. 1.)

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(Received 15th July 2008 ; accepted 20th October 2008)

Abstract. This work is the first part of a series of studies, which introduces the methodological possibilities of coenological and zoogeographical indication and – following the climate, vegetation and elevation zones – the pattern-describing analysis of the main Oribatid sinusia of the world explored till our days.This current work is a case-study, which displays the comparison of 9 examination sites from 3 different geographical locations. On each location, three vegetation types have been examined: a plain rain-forest, a mossforest and a mountainous paramo. Analyses are based on the hitherto non-published genus-level database and coenological tables of the deceased János Balogh professor. Occurrence of 18 genera is going to be published as new data for the given zoogeographical region.
Keywords: *Oribatid mites, rainforest, mossforest, paramo, Costa Rica, Brazil, Papua New-Guinea, similarity pattern, coenology, community, assembly*

Introduction

Oribatid mites of the tropical regions had been almost completely unknown for science before 1958. With the general use of the Berlese-funnel, systematic collections started at that time, which resulted in the collection of hundreds or thousands of species. However, the majority of these samples are unprocessed till these days. Professor J. Balogh and his fellow-workers had to realize, that the description of all the species living there is an impossible task.

And even if the recognizable morphological kinds of the samples will be described with decades of monumental work by the practices of formal describing taxonomy and according to the rules of nomenclature, the biological and coenological information content of these would still remain hidden. However the material extracted with the Berlese-Tullgren device informs us not only about the presence and morphological diversity, but also about the species' abundance and dominance as well. Moreover it has

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become clear that this material together, as it was brought in front of us, contains a heap of such kind of information, which would be impossible to read from single species or from their constitutional characteristics. The samples collected this way are suitable for zoocoenological examinations. This observation led Humboldt to the recognition of „basic forms" (Grundformen) and later to the revelation of formations, which means the structure that can be found in plant associations without the exact knowledge of species. The emphasis here is on the „visibility" of the vegetation, because the recognition of biological communities began with the sight of the flora: vegetation is a „landscape element". Animal communities – apart from some exceptions – live hidden in the vegetation. But the Berlese-device concentrates and makes them visible.

Supported by the Division of Natural Sciences of UNESCO, and then with the help from other sources and by decades-long, even currently running work, J. Balogh and his followers (the senior authors of this paper among them) have been taking surveying collections in the tropical rainforest soils of Africa, South-America, South-East-Asia, New Guinea and of the islands of the world's oceans. The picture drawn by the experiences gathered on three continents shows a disastrous destruction of natural vegetation, first of all tropical rainforests. If soil zoology wants to keep pace with this devastation, such a new, global research plan has to be made which provides before long a globally perspicuous picture of Oribatid fauna. Obviously, traditional museological and phenetic taxonomical methods in themselves are unsuitable for this task. In the view of collection technique, Berlese-device provides a unique advantage. Based on the available results of explorative field work the time has come to explore the hidden zoocoenological information in the structure of Oribatid-associations and thus pave the way towards the forming of exact, academic hypotheses.

It would have been impossible to survey the whole tropical zone of the Earth. This is why two broad, representative belts have been chosen. The first of them follows the range of Andes, spreads from the Tropic of Cancer to the Tropic of Capricorn and not only covers the range of Andes, but also the broad tropical belt lying to the East of them. The 6000 m high ridge of Andes forces the clouds arriving from the direction of the ocean to ascend. Thus, on the Eastern slope of the mountain, zonations of luxuriant mountain forest and mossforest can be found. It has been planned that collections between the two Tropics, at different latitudes would be made through the Andes in zonal transects, and also in a regional transect which crosses the whole continent. The tropical zone of South America contains a mountain which is independent from the range of Andes: the Serra do Mar, and there's also a parallel range of Serra do Mantiguéra which lies to the Northeast of Sao Paolo on the coast of the Atlantic Ocean. It lies on the most densely populated area of Brazil; therefore 95 % of its vegetation has already been destroyed. Recently, authorities try their hardest to save what is possible for science. This is the reason why the Hungarian research group also involved this region into their examinations. Their collection spots were located in 5 broad transect belts of the Neotropical Region. The first belt follows by and large the 20., the second the 10. grade of Northern latitude, the third the Equator, the fourth and fifth the 10. and 20. grade of Southern latitude (see sketch-map).

The other representative belt begins at the tropical coasts of Australia by Rockhampton, crosses Great Dividing Range and breaks 5 degrees to the South of Equator in Papua New-Guinea, at Owen Stanley Range. No other greater mainland can be found to the north of the Equator in this belt. Collections have been made at two

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important areas to the East of the Australia-New Guinea line: in New Caledonia (north from the Tropic of Capricorn) and in the Hawaii-islands (south from the Tropic of Cancer). As it is known, the subduction of Australian Plate is to the east from New Caledonia, in the „Hebride-ditch". These two area belts are connected with their common Gondwana-past. Plates which lie on the present area of South-America represent the Western, and those of New-Caledonia and New-Guinea represent the Eastern territories of Gondwanaland.

These two area-belts which were selected for examination are not only connected by their common Gondwana-past, but also by the spread of two plant genera: Araucaria and Nothofagus. Both connect Neotropical Region with Notogaea in the shape of a giant „U". The South-American branch of this „U-shape" follows by and large the range of Andes, but it is split into two parts around the Tropic of Capricorn and forms the Serra do Mantiguera- Serra do Mar twin mountain ranges in South Brazil. The two branches of the giant U-shape meet at Antarctica. The continuity is testified here partly by fossil remnants and partly by Araucaria and Nothofagus fossil pollen. The Eastern branch of the U-shape lies on the New-Zealand- New Caledonia – New-Guinea line and ends below the Equator. Araucaria formed great forests in South-Brazil, Argentina and Chile, but most of them are destroyed by these days. However, even nowadays there are great stands in New-Guinea. The gene centre of Araucaria is in New-Caledonia, 13 of the known 19 species live here (Schmidt, 1981). The range of Nothophagus is roughly identical to Araucaria. Great populations can be found on the temperate parts of Argentina and Chile and in New-Zealand. Its gene centre is in New-Guinea, where significant part of the known species lives.

The areas selected for examination were further narrowed down by concentrating our collections mainly to the humid tropics. Most of our collection sites had been on locations where vegetation formed closed forests due to abundant precipitation. These areas were: zones of tropical rainforest, tropical mountain forest and tropical mossforest, and even grass areas above forest threshold.

Beside vegetation types, the composition of Oribatid species is decisively influenced also by macroclimatic conditions. The data of Walter and Lieth Climatic Atlas, the so-called climatic graphs provide sufficient information on the annual or even daily changes of the macroclimatic conditions of the examined sites. Walter and Lieth discloses such kind of data on more than 10,000 locations of the Earth. Data are disclosed by continents and inside the continents, by climatic zones. This way, direct or indirect, but adequate data can be found for any location of the Earth. The vegetation type and the applying climatic graph can provide such kind of particular information, that the generic composition of the Oribatid sinusium can be described with great probability – even if we collect Oribatids on the site for the first time.

The Berlese-samples had been recorded separately, according to vegetation levels (substrate types). Main substrates were: soil, litter, moss, hanging litter, bark, epiphytes, canopy; and various transitions or combinations of these. Oribatids which were collected in the identical level (substrate-type), identical locality and extracted with Berlese-Tullgren device have been considered as units of examination. This material has been called Oribatid-sinusium. In one level, generally 2-10 – and in some exceptional cases even more – parallel materials have been collected. These were extracted and examined separately, but the group of parallel materials collected from one locality has been considered as sinusium. The number of parallel samples of sinusia was intended to be chosen in such a way that it should represent the whole of the habitat

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properly, so more prevalent substrates (and such, also sinusia) have been represented by more sample units. Between 1965 and 1995, there have been 20 collecting journeys altogether in those representative belts with the purpose of collecting Berlese-samples available for coenological analysis. The sinusia collected from different areas have been distributed as follows:

Neotropical Region	
Cuba	80
Costa Rica	141
Ecuador	207
Brazil	386
Australia and Oceania	
Tropical Australia	82
New-Caledonia	133
New-Guinea	394
Hawaii Islands	95

Later on, these were supplemented with further samples:

According to the climatic classification of Walter and Breckle (1983), the area of rainforest clusters around Sao Paulo and Rio de Janeiro belongs to the group of extraequatorial tropical rainforests. However, relatively far from the Equator, in the zone of seasonal rainfalls – Zonobiome II (ZB II) – due to the effect of abundant rainfalls coming from the direction of the Atlantic Ocean, a perhumid Subzoobiome (sZB II/e I) is formed on the shore. This is also called tropical rainforest for simplicity.

The daily fluctuation of the temperature in the typical equatorial rainforests – especially in the soil – is quite negligible, and the mean annual temperature hardly changes. The situation is similar in the perhumid Subzoobiome of Zonobiome II. In the Orobiomes - which join to the tropical Zonobiomes – or tropical mountains, the annual mean temperature changes by 0.5-0.6°C in average with every 100 m increase in elevation above sea level. The fluctuation in the temperature has a decisive influence on the fauna of tropical rainforest and also to their soil fauna. Species with small tolerance – the so-called stenotherm species – can survive only in flatland or low-mountain jungles. The upper limit of their prevalence is – depending on the geographical latitude of the area – about 1000 m elevation above sea level. The next vertical prevalence limit is around 1700-2000 m, where the tropical rainforest is replaced by tropical mossforest. The diversity of the soil fauna is the greatest in this region. Above the tree line, the fauna changes completely, diversity sharply decreases and species with extreme cold tolerance appear.

Objectives

In this present section of our examinations, we set the objective to clarify the methodological possibilities of biological indication and the information content of the coenological data matrices by an appropriately chosen indication case study. We also set the aim to apply univariate indicators and to exploit the possibilities of multivariate coenological pattern analysis.

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In this study, we attempted to answer the following indicational questions (exploring the following phenomena):

Taxonomical questions and their indicators

1. Is the average species number of those genera where the present individuals belong to characteristic to a habitat or not? (Indicator: The arithmetic average, empirical variance and relative variance of the species number of Oribatid genera present in the examined habitat weighted by constancies).
2. Is the information on the extent of taxonomical isolation of present genera characteristic for a habitat? (Indicator: The average number of genera, its empirical variance and relative variance of the family number of Oribatid genera present in the examined habitat).

Zoogeographical questions and their indicators

3. Is the information on the worldwide spread of species belonging to the present genera characteristic for a habitat? (Indicator: presence in the examined habitats of the species belonging to the examined Oribatid genera in the main zoogeographical regions with the method of "simple voting".)

Question of spatial heterogeneity and its indicator

4. Is the information on the extent of difference in the genus composition of the different soil samples collected from the same habitat characteristic for a habitat? (Indicator: the average pairwise dissimilarity of samples collected from the same habitat (with distance functions calculated for the presence of genera), as a coenological heterogeneity indicator).

Coenological (multivariate) questions and their indicators

5. Is the similarity pattern of genera of different habitats informatory on the type and/or geographical location of the habitat? (Indicator: the multivariate similarity pattern of Oribatid community of habitats).
6. Is the similarity pattern of Oribatid genera in soil samples collected from different habitats informatory on the type and/or geographical location of the habitat? (Indicator: the multivariate similarity pattern of Oribatid community of individual soil samples of habitats).
7. The type or the geographical location of a habitat plays bigger role in the formation of the present Oribatid fauna? (Indicator: linear regression model).

Review of literature

Berlese and Grandjean

The establishment of Oribatology in the last 100 years can be linked to two great persons: A. Berlese and F. Grandjean. If we look at the dates of the first and last publications of these two persons (Berlese: 1880-1923; Grandjean: 1928-1974), we can recognize the beginning and the end of two 50-year long eras. The first period, among many other things, provided us with an outstanding extraction method: the Berlese

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device. This method – regarding its significance – can be compared to the invention of the microscope. It led us to the exploration of a new, unknown microcosm: the mezofauna of the soil and thus, the establishment of a new field of science: soil zoology. In the second era, a congenial scientist – F. Grandjean – not only spotted the most subtle structures under his microscope, but also unravelled the principles of hundreds of millions years of evolution. It is not so self-explanatory that these two aspects do not exclude, but on the contrary, they supplement each other. If the subject of the branch of biological discipline is the existing, given living organism in its timeless existence (esse, being, das Sein), then we speak about the present, current branch of discipline. This is the aspect of Berlese's work of life. And if the subject of the biological discipline is the genesis, past, evolution of the living organism, in other words, its existence through the time (fieri, becoming, das Wesen), we speak about a genetic branch of discipline. This is the essence of Grandjean's work-life. To understand the structure and functioning of biosphere, we have to learn the components of the whole system in a form they exist today. Then we have to learn their evolutionary past, where they originate from. This is how the two aspects can be linked to each other. Problems arise with the fact that these two aspects work with different objectives. Those who study the Oribatid fauna of the world, the Oribatid assemblies in the ecosystems, want to give little but important information on as many species as possible, because such kind of differentiating characteristics are sufficient for the recognition of the building elements of these assemblies. And those, who deal with the evolution of Oribatids, want to obtain a tremendous number of data - which are important from the aspect of evolution - but only on a little number of species. Since the Hungarian research team dealt with the examination of Oribatid assemblies and their spatial distribution of Oribatids, they represent the first group of oribatologists.

Further international studies

The „first wave" of research started in 1958. By that time we have to differentiate between preliminary and general collections. On the fist place we mention the exemplary work of Aoki et al. in Japan and Eastern Asia. The subantarctic research of Wallwork is of general significance, however his work in Ghana and his further African works can be considered only as preliminary studies. In the „second wave" which started in the late sixties, - among many others - the works of Engelbrecht (1969) and his fellow researchers in South Africa; Corpus-Raros (1979) on the Philippines, Lee (1980) in Australia, Pérez-Inigo and Baggio (1980) in Brazil, Bhattacharia et al. (1972) and Haq (1980) can be considered to be of general importance.

Hammer and Beck

The fact that 90 % of tropical Oribatid species were unknown until the late fifties, made coenological examinations impossible, however there have been many attempts to reach this goal. Hammer (1958-1962) published four fundamental works on the Oribatids of the South American Andes, partly even from tropical areas. Starting from Peru, down to the Tierra del Fuego he collected almost 50,000 Oribatid specimens. He identified the whole collection and found 466 species, of which 324 were unknown. Thus he laid down the basics of the description of the South-American Oribatid fauna. He also started the coenological processing of the material, but the taxonomic work exhausted him in such an extent that he hadn't had the time to complete this work. The

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fundamental work of Beck (1963) has a similar significance. He examined mites in only one region of Peru, in the main vegetation zones of the Andes. He completed 300 Berlese-collections with more than 350,000 specimens. A significant part of the examined material belonged to the Oribatids. He made important statements primarily concerning the „autoecology" of the species, but the huge number of undescribed species made coenological analysis impossible. The holistic point of view is characteristic both to Hammer and Beck. They applied their examinations not only to individual species but to the whole Oribatid community. Unfortunately, there has been a fundamental difference between the point of view of Hammer and Beck. Hammer was driven primarily by biogeographical problems; he wanted to learn the regional distribution of Oribatids. This is why he marked his collection spots in a range of thousands of kilometers from Peru to the Tierra del Fuego (Land of Fire). Beck based his studies on the different elevation zones of vegetation, so he studied the zonal distribution of species. It is obvious, that a world-wide study requires both of them, and the Hungarian research team planned an examination exactly of this kind. In their plan, both regionality and zonality obtained their roles.

General findings of the coenological Oribatid examinations

The greatest experience of a first look on an extracted Berlese-material is that unevenness can be found in the Oribatid assemblies. Some species appear in great numbers, while only a few can be seen from others. The second experience has been that if we repeat the collection over and over again, certain species appear constantly. Species with great numbers are called dominant, and the ever-appearing are called constant species. These two characteristics can be experienced often by the same species, so these are called constant-dominant species. According to the third experience, there are some species, which live only in certain kinds of Oribatid assemblies; they can be collected only in that combination of species. These are called character species. And according to the fourth experience, certain Oribatid species – however, apparently there's no direct connection among them – always appear together in the Oribatid assemblies.

New results of Oribatid bioindicational studies

Analysing the suitability of Oribatids for indication purposes, many authors point out their long life-span, slow growth and slow dispersion ability. Based upon these characteristics, Oribatids can be used as strong indicators of their environment (Gulvik 2007). Current bioindicational studies based on Oribatids use just the measures of Oribatid abundance or species richness at most. A significant part of these studies are directed to analyse anthropogenic effects. According to Niedbala (1990), every kind of human interference decreases the abundance and species richness of the mites. Oribatids are thought suitable – though in different extent – for bioindication purposes in respect of chemical pollutions according to works of Andres 1999, Braman 1993, Liiri 2002, Miglorini 2005, Prinzing 2002, Zaitsev 2001, Seniczak 1995, Skubala 2004, Stamou 1995, Steiner 1995, Straalen 2001 and Osler 2006. Many authors refer to the role of moisture and organic material content of the soil in affecting the abundance of Oribatids (Braman 1993, Siepel 1996, O'Lear 1999, Kovac 2001, Tsiafouli 2005, Altersor 2006); however their statements concerning the direction of these effects are contradictory. This is not surprising, since different communities had been examined. Based on

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surveys of different approaches, effects of human indicated changes in the structure of vegetation (grazing, agricultural cultivation, forestry etc.) seem to be clear (Migliorini 2003, Lindo 2004, Altesor 2006, Berch 2007, Cole 2008), since disturbances of vegetation result in lower Oribatid abundance. We think however that the role of anthropogenic effects can only be entirely understood if the reasons and patterns of naturally induced variability in community structure were explored first, since these can be considered a reliable reference in the evaluation of anthropogenic disturbances. Examining the effects of different vegetation types and floral succession stages, many authors (Noti 1996, Salmon 2006, Osler 2001 és 2006, Coleman 2008) describe high Oribatid abundance and species richness in natural or almost natural habitat types. These authors also point out that the role of definite plant species, substrate types and microhabitats does not seem to be significant form the aspect of the composition of Oribatid communities (Kaneko 2005, Franklin 2005, Fagan 2006, Lindo 2006), though contradictory data also exist (Coulson 2003). By analyzing African Oribatid communities, Noti (2003) attributed the most significant effect to vegetation type, followed by moisture content, then organic material content, C/N ratio and N-content in respect of species richness.

Materials and methods

Examined areas and their vegetation

This work is intended to be the first part of a series of studies consisting of several parts. In this study series, we want to introduce the main Oribatid sinusia of tropical areas as per climatic, vegetation and elevation zones. According to the holistic approach, we start from the whole and proceed towards the smaller parts. As a first step, we examined the similarities between the Oribatid sinusia of the Neotropical Region and the Notogaea. Stemming from the fact that the vertical stratification of Oribatid fauna follows the vegetation zones, we took samples for examination from 3 elevation zones:

1. tropical rainforest
2. mossforest
3. paramo

In the study, we disregarded the mountain forest zone, which can be found between the zones of tropical rainforest and mossforest, because the determination of its borders is quite uncertain. To avoid transitions, Berlese-samples of tropical rainforest have been selected from 200 m elevation above sea level, close to the forest border. 2 ideal transects were set for representative sampling. The first one crosses Andes at Costa Rica, at the 10. degree of latitude, from coast to coast. The second one starts from Papua New-Guinea, from the valley of Fly River at the 4. degree of Southern latitude, and goes up to the 4000 m high ridge of Mt Wilhelm. As an amendment, samples were also taken near to the 23. degree of Southern latitude in an additional transect, crossing Serra do Mar and Serra do Mantiguera. This transect has been set because 200-300 years ago there's been a belt of dense tropical rainforest – which even exceeded Amazonia in biodiversity – in the most densely populated area of Brazil, along the line marked by Sao Paulo and Rio de Janeiro. Almost 95 % of these forests have been devastated, but it could have been hoped that the rainforest spots reserved the original soil fauna – at least

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partially. 82 representative samples have been collected on 9 spots of the three transects. The spatial distribution of these samples and the abbreviation of individual sites can be seen in the next table:

	Tropical rainforest	Mossforest	Paramo	
Costa Rica	RC: 10	MC: 10	PC: 10	30
Brazil	RB: 7	MB: 6	PB: 10	23
New-Guinea	RN: 10	MN: 9	PN: 10	29
	27	25	30	82

Taxonomical processing of the collected material

Oribatid mites are one of the land animals with the highest population density. Their abundance among tropical conditions can exceed one million individuals per square meter, but even among the conditions of the temperate zone, their density is 50,000-500,000 per square meter, while in agricultural areas this measure is around 25,000 in average (Coleman et al. 2004). Their numbers are also significant in treebark (Erdman 2006) and foliage (Winchester 1997, Behan-Pelletier and Walter, 2000). Using 16, 32 or more Berlese-devices during many years in a worldwide sample collection we get such an extreme amount of individuals, which is impossible to identify completely. Given such a worldwide programme, set forth by the Hungarian research group, the processing of the entire material is even more inconceivable. Thus we decided to render the second level of the system, the genus, as the base of the processing. Members of our research group – formerly led by János Balogh – knew well before the initiation of coenological analysis that the genus could be the taxonomical level which can bear valuable information for an examination of such a magnitude. Using species as a category at the current level of Oribatid taxonomy would be an entirely hollow task, since the decisive majority of the described morphological species apparently have no valuable ecological or evolutional information content. Thus, it is not a coincidence that the work of Hammer and Wallwork (1979) on the connection between the worldwide distribution and continental migration of Oribatids is based on generic distribution from the beginning to the end. Most biogeographical and many ecological studies do so in connection with other groups of animals. This is not a coincidence, but a consequence originating from the past and (unfortunately) the current practice of species description. The interpretation of species of the phenetic descriptive taxonomy is under the subjective approach of the describing taxonomist, since there are no uniform rules in this respect. If an individual is found to be different from the formerly described species in 2-3 seemingly independent morphological characteristics, then it is usually automatically considered as a new taxon. Descriptive taxonomists are interested in the propagation of the number of nominal species, and in accordance with the aforementioned, their activity is not subjected to strict scientific critics. Thus the question whether a species described correctly from the aspect of nomenclature can be considered as a new inherent biological entity or not, usually does not even emerge. Resulting from quite understandable reasons, the modus operandi is eclectic even by the description of these nominal species. Conspicuous, easily identifiable species have been preferred. In large genera with many species, much less species is described because it requires more work and unproductive intellectual efforts. Early auctors strove to briefness, listing of the differentiating characteristics by the description of species, thus

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the more general characteristics; really important biological strains were put forward during the description of genera. As a consequence, it is quite possible that slightly different species from the same genus represent only the variability within the biological species. Obviously, to unveil this, is an important scientific task.

The generic exploration of the Oribatid mites of the world among the current conditions seems to be sufficient from the aspect of the valuable biological research, and the genera tend to represent real biological phenomena more likely than the infinite nominal species.

Despite these grave scientific problems, coenological, zoogeographical and ecological studies are compelled to use the classic morphological, descriptive taxonomical works as a base until molecular techniques capable of the definite identification of kinds do not become simple, fast and cheap enough for the routine processing of large materials.

Assembling coenological tables

The base table for our analyses was the 82 individual soil samples from the 9 examined habitat (3 habitat types of 3 areas) containing 111 Oribatid genera. For the various analyses, we created task-oriented assemblies from this base table. We created the constancy table of the genera of the 9 habitats, where the constancy values of the individual genera have been estimated by the relative occurrences in the related samples (*Table 1*). As a complementary data source, we created the occurrence table of the same genera in the zoogeographical regions based on the monography of Balogh and Balogh 1992, complemented with own, new data (*Table 2*). Using the same monography as a reference, we looked up the number of species of the genera, and the number of genera of the families which we used as complementary information for the analyses. Based on all of the individual samples, paired all samples with all other samples we created the similarity matrix of our data using multiple distance functions. In this current publication, Euclidean distance, PHI coefficient and Jaccard function have been used.

Methods for analyzing coenological tables

We created the indicators marked by our objectives from our coenological tables, and analysed the values of these indicators in the different sites. As a taxonomical indicator, we calculated the average number of species of genera found in individual habitats weighted by the constancies (and also its empirical and relative variance), and the average number of families of the same genera (and variances). As a zoogeographical indicator, we introduced the method of "simple voting", where the occurring genera give one vote for those geographic regions, where at least one of their representatives can be found. Occurrence of one genus in one region means 1 vote for the region in question. Votes of genera of a certain site given for different regions are represented as a % proportion of the total votes of these genera, what is characteristic for the habitat. We examined the constancies of genera on individual habitats, and the generic heterogeneity of the samples. The measure of heterogeneity was calculated by pairing every sample with all the other samples within a site. Based on the presence-absence data of their genera, we created their distance matrices and calculated the average of the distance values. After the evaluation of univariate indicators we analysed the similarity patterns of the sites with Non-Metric Multi-Dimensional Scaling (NMDS) and Hierarchical Cluster Analysis (HC-UPGMA) using multiple distance functions, then

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we conducted these analyses on the tables of individual elementary samples. We applied multivariate regression modelling in order to further evaluate the results of multidimensional pattern analysis and to test our hypotheses. Gathered results have been evaluated collectively and compared with literature findings, then the examined habitat has been characterized by these results.

Results

Results of taxonomical processing of field samples

By the taxonomical processing of field samples we determined the occurrence data of the found genera, which have been made comparable by representing them in constancy %. Results are summarized in *Table 1*, where we marked the dominant genera (with mass occurrence) by underlining beside the constancy data. High constancy value and underlining together marks constant-dominant genera. Most rigorously constant-dominant genera are the **Xenillus** and the **Plasmobates** in the Brazilian mossforest, the **Oppia** in the New-Guinean mossforest, the **Allonothrus** in the Costa-Rican rainforest and the **Rostrozetes** in both the Costa-Rican and New-Guinean rainforests. According to our data, the **Eremulus, Eohypochthonius** and **Teleioliodes** genera are characteristic for the rainforests, the **Oppia** genus for the mossforests, and the **Scheloribates** genus for the paramos. Geographic characteristics can only be observed by the **Anderemaeus** genus for Brazil and the **Dolicheremaeus** genus for New-Guinea. We couldn't find a single genus characteristic for the American continent. **Malaconothrus** and **Microtegeus** genera could be found on all sites (general prevalence).Based on the monography of Balogh and Balogh 1992, occurrences of genera found by us in the zoogeographical regions of the world are summarized in Table 2. According to our own data, in comparison with the aforementioned monography, occurrence for the relating zoographical region of 18 genera can be published as new data, these are marked underlined. Based on the data of the table it is clearly visible that the **Malaconothrus** and **Microtegeus** genera (observed by us on all sites) are truly cosmopolites, similarly to the **Dolicheremaeus** genus, which we found characteristic to New-Guinea. However, the **Anderemaeus** is known only in Notogaea and the Neotropis. The **Scheloribates, Eremulus, Eohypochthonius, Rostrozetes, Plasmobates** and the **Oppia** genera are cosmopolites. The **Teleioliodes** genus can be considered prevalent in Gondwana. The **Xenillus** is known from Holarctis, Notogaea and Neotropis, and the **Allonothrus** genus is circumtropical. These data point out, that members of genera with very wide prevalence from purely geographical aspect are quite discriminative in respect of habitats, and thus can be informatory for us.

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Table 1. *Constancy % of Oribatid genera found on individual sites*

GENUS	MB	MC	MN	PB	PC	PN	RB	RC	RN
Achipteria		26.67							
Acutozetes									20.00
Aeroppia								23.08	
Afronothrus								38.46	13.33
Allonothrus								100.00	
Allozetes								7.69	13.33
Anachipteria					31.82				
Anderemaeus	83.33			10.53			28.57		
Aokiella									6.67
Arceremaeus								30.77	
Archegozetes								30.77	6.67
Basilobelba								7.69	
Bornemiszaella								23.08	
Brazilozetes							14.29		
Camisia				31.58	4.55				
Carabodes		46.67						23.08	
Caudameolus							28.57		
Cavernocepheus								7.69	
Cepheus		13.33			40.91			15.38	13.33
Ceratokalumma? =Achipterina	66.67								
Ceratoppia					4.55				
Ceratorchestes	33.33	33.33			11.11		28.57	38.46	
Ceratozetes					13.64				
Charassobates	33.33						28.57	15.38	
Compactozetes			33.33						
Crotonia	66.67	80.00		15.79	47.62				
Cubabodes					25.00			7.69	
Cultroribula						4.35			
Cyrthermannia								30.77	73.33
Dampfiella		26.67			27.27		28.57	7.69	13.33
Dendrohermannia									40.00
Dolicheremaeus			33.33			21.74			80.00
Edwardozetes				5.26	11.11	26.09			
Eohypochthonius							71.43	38.46	33.33
Epilohmannia				5.26			71.43	53.85	6.67
Eremaeozetes	16.67			10.53			28.57	61.54	66.67
Eremobelba	66.67			21.05			57.14	15.38	86.67
Eremulus							57.14	23.08	86.67
Eupterotegaeus	50.00	86.67			63.64				
Eutegaeus	33.33			5.26					
Furcobates		20			13.64				
Fuscozetes				5.26	50.00				
Galumna						4.35			
Galumnella									6.67
Gressittolus									13.33
Gustavia					22.22				

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GENUS	MB	MC	MN	PB	PC	PN	RB	RC	RN
Hammerabates						34.78			
Hamotegaeus					4.55		28.57		
Haplozetes								7.69	
Hardybodes									26.67
Hermannia	33.33			5.26			57.14		80.00
Hermanniella	16.67	86.67			59.09		71.43	30.77	20.00
Heterobelba	66.67	13.33		5.26	12.50		85.71	7.69	
Heterozetes			11.11						
Idiozetes									20.00
Indoribates									6.67
Lamellobates							42.86	7.69	
Liacarus		46.67							
Licneremaeus								15.38	
Limnozetes				5.26					
Malacoangelia								16.67	60.00
Malaconothrus	50.00	60	22.22	42.11	31.82	21.74	71.43	92.31	73.33
Masthermannia								15.38	53.33
Microtegeus	83.33	33.33	22.22	26.32	11.11	13.04	71.43	53.85	40.00
Microzetes				21.05					
Mochlozetes				42.86				30.77	
Mycobates				5.26	4.55				
Nanhermannia	83.33	60		63.16	50.00		71.43		28.57
Neoribates									53.33
Nixozetes									33.33
Nodocepheus	50.00	26.67		15.79	11.11			7.69	
Nothrus	16.67	46.67		15.79	28.57				
Oppia	83.33	93.33	100.00						
Oribatella	66.67	26.67						30.77	33.33
Oribatula				57.89					
Otocepheus									6.67
Oxyamerus									40.00
Pelops			33.33		31.82	4.35		15.38	
Peloribates	66.67			47.37			42.86	7.69	46.67
Phauloppia				5.26					
Pheroliodes	50.00		11.11	21.05	16.67	21.74	71.43	7.69	
Plasmobates	100.00	60.00		26.32	52.38		71.43	30.77	66.67
Plateremaeus								7.69	33.33
Platynothrus		53.33	66.67	5.26	36.36	21.74			
Protoribates					27.27	4.35	28.57	15.38	
Pseudotocepheus								23.08	
Pteroripoda		80.00							
Reductobates			11.11			17.39			
Rhynchoppia					23.08				
Rhynchoribates	50.00	20.00					28.57	23.08	
Rioppia	16.67						14.29	15.38	
Rostrozetes	100.00	60.00		26.32	54.55		71.43	100.00	100.00
Scapheremaeus		80.00		36.84	36.36	8.70			
Scheloribates				73.6	72.73	26			
Sphaerochthonius								7.69	
Sternoppia								7.69	

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GENUS	MB	MC	MN	PB	PC	PN	RB	RC	RN
Suctoribates	83.33			5.26					13.33
Tectocepheus	33.33	26.67		5.26	45.45	8.70			
Tegeocranellus	66.67						57.14		
Tegoribates				5.26	36.36				
Teleioliodes							71.43	38.46	40.00
Tentaculozetes									20.00
Trichoribates					4.55				
Truncozetes								76.92	
Tuberemaeus						4.35			60.00
Williamszetes				21.05					
Xenillus	100.00			10.53	27.27		85.71	15.38	
Xiphobelba									80.00
Xylobates						8.70			93.33
Zetorchestes									26.67
Zygoribatula	16.67				4.55	4.35			

Table 2. *Number of species and presence characteristics of the examined genera according to Balogh and Balogh (1992). In the collected samples 18 genera were new to the fauna of the given zoogeographical region compared to this monography (bold underlined).*

GENUS / (Family)	ET	NO	HO	OR	AN	NE	Species number
Achipteria	1		1	1		**1**	47
Acutozetes		1		1			5
Aeroppia						1	11
Afronothrus	1	1		1		1	5
Allonothrus	1	1		1		1	12
Allozetes	1	1	1	1		**1**	8
Anachipteria	1		1			**1**	19
Anderemaeus		1				1	8
Aokiella		**1**		1			2
Arceremaeus						1	4
Archegozetes		1		1		1	5
Basilobelba	1	1		1		1	13
Bornemiszaella						1	3
Brazilozetes						1	3
Camisia	1	1	1	1	1	1	39
Carabodes	1	1	1	1		1	130
Caudameolus						1	1
Cavernocepheus						1	2
Cepheus	1	**1**	1			1	60
Ceratokalumma? =Achipterina	1	1				**1**	4
Ceratoppia	1		1	1		1	17
Ceratorchestes						1	4

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GENUS / (Family)	ET	NO	HO	OR	AN	NE	Species number
Ceratozetes	1	1	1	1	1	1	89
Charassobates						1	7
Compactozetes		1					4
Crotonia	1	1				1	2
Cubabodes						1	5
Cultroribula	1	1	1	1		1	34
Cyrthermannia	1	1	1	1		1	12
Dampfiella		1		1		1	14
Dendrohermannia		1					1
Dolicheremaeus	1	1	1	1	1	1	105
Edwardozetes		1	1		1	1	8
Eohypochthonius	1	1	1	1	1	1	6
Epilohmannia	1	1	1	1		1	44
Eremaeozetes	1	1		1		1	17
Eremobelba	1	1	1	1	1	1	38
Eremulus	1	1	1	1	1	1	27
Eupterotegaeus			1			1	9
Eutegaeus		1				1	11
Furcobates						1	1
Fuscozetes			1			**1**	10
Galumna	1	1	1	1	1	1	207
Galumnella	1	1	1	1		1	14
Gressittolus		1					2
Gustavia	1	**1**	1	1		**1**	18
Hammerabates	1	1		1			4
Hamotegaeus						1	4
Haplozetes	1	1	1	1		1	11
Hardybodes		1					1
Hermannia	1	**1**	1	1		**1**	51
Hermanniella	1	1	1	1		**1**	32
Heterobelba	1			1		1	13
Heterozetes		**1**	1	1			6
Idiozetes		**1**		1			2
Indoribates		**1**		1			2
Lamellobates	1	1	1	1		1	11
Liacarus	1	1	1	1	1	1	80
Licneremaeus	1	1	1	1	1	1	18
Limnozetes			1	1		1	17
Malacoangelia	1	1	1	1		1	4
Malaconothrus	1	1	1	1	1	1	51
Masthermannia		1	1	1		1	5

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GENUS / (Family)	ET	NO	HO	OR	AN	NE	Species number
Microtegeus	1	1	1	1		1	22
Microzetes			1			1	13
Mochlozetes	1		1			1	6
Mycobates			1			1	17
Nanhermannia	1	1	1	1		1	29
Neoribates		1	1				19
Nixozetes		1		1			5
Nodocepheus	1	1				1	7
Nothrus	1	1	1	1	1	1	54
Oppia	1	1	1	1		1	16
Oribatella	1	1	1	1		1	113
Oribatula	1		1	1		1	80
Otocepheus		**1**		1		1	20
Oxyamerus		1	1	1			6
Pelops	1	1	1	1	1	1	80
Peloribates	1	1	1	1	1	1	84
Phauloppia	1	1	1	1	1	1	27
Pheroliodes	1	**1**				1	3
Plasmobates	1	1	1	1	1	1	19
Plateremaeus	1	1	1	1		1	19
Platynothrus	1	1	1	1	1	1	31
Protoribates	1	1	1	1	1	1	50
Pseudotocepheus	1	1		1		1	21
Pteroripoda						1	1
Reductobates		1					3
Rhynchoppia		1				**1**	1
Rhynchoribates	1			1		1	22
Rioppia						1	2
Rostrozetes	1	1	1	1	1	1	40
Scapheremaeus	1	1	1	1	1	1	50
Scheloribates	1	1	1	1	1	1	200
Sphaerochthonius	1	1	1	1	1	1	10
Sternoppia						1	12
Suctoribates	1	**1**		1		1	3
Tectocepheus	1	1	1	1		1	37
Tegeocranellus	1	1	1	1		1	8
Tegoribates	1		1			1	9
Teleioliodes	1	**1**				1	3
Tentaculozetes		1					1
Trichoribates	1		1			1	55
Truncozetes						1	2

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GENUS / (Family)	ET	NO	HO	OR	AN	NE	Species number
Tuberemaeus	1	1		1			26
Williamszetes						1	2
Xenillus		1	1			1	74
Xiphobelba		1		1			5
Xylobates	1	1	1	1	1	1	39
Zetorchestes	1	1	1	1	1	1	15
Zygoribatula	1	1	1	1	1	1	106

Results of numerical evaluations

Taxonomical indicators

From ecological and faunagenetical aspects, we considered the species number of the genera and the genus number of the families as appropriate for indication purposes among the information stemming from the taxonomical position of individual taxa. The number of subtaxa of a taxon can show the evolutionary success of the taxon, the extent of adaptive radiation, but it can also bear information on the evolutional age or even about the geographical spread of the taxon. The subtaxon number of the higher hierarchy levels obviously stems from a more distant geohistorical past, than that of lower hierarchy levels. For taxonomic indicators, we calculated the average of number of species of genera found on individual habitats weighted by constancies (and the empirical and relative variances), and the average genus number (and variances) of the related families (*Table 1).* In respect of Costa Rica, Brazil and New-Guinea we found that ascending from the tropical rainforest to the top of the mountain (to the direction of mossforest and paramo vegetation) the average species numbers of genera show a gradual growth. By analyzing the empirical variance of this indicator, it can be stated that the least variance can be found at the level of rainforest. This means that members of genera with higher number of species live in higher altitudes, the highest average number of species can be found in the Costa-Rican paramo, and the least in the Costa-Rican rainforest.

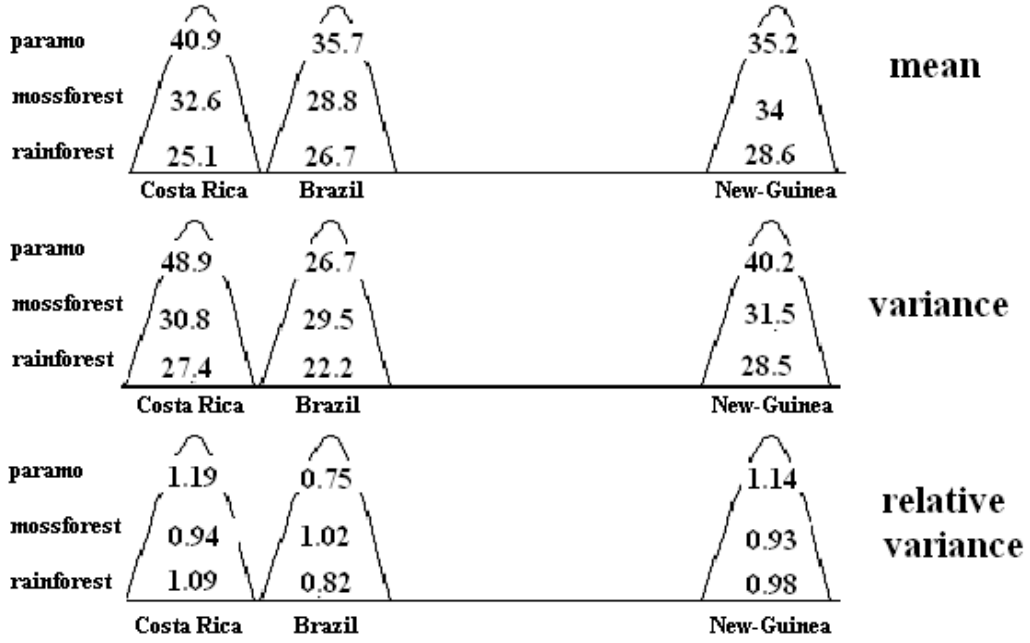

Figure 1. *Average number of species and variance of genera of individual habitats*

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A similar analysis can be conducted by evaluating the average genus number of the families which the genera belong to (*Table 2*.). This indicator has been implemented to show the "taxonomical isolation" of the related genus. Figure shows that in all three examined areas, representatives of families with the highest genus number lived in the mossforest, and those with the lowest genus numbers in the rainforest. Compared to the rest of the habitats, mossforests' genera can be characterized with the "most populous" family. Paramo always stood closer to the mossforest in this respect. Furthermore it can be stated that both the empirical and relative variances of this indicator are highest in mossforests. Comparing the areas, we can see that the average values in New-Guinea are slightly higher than those on the American continent. Lowest value can be found in the Brazilian rainforest.

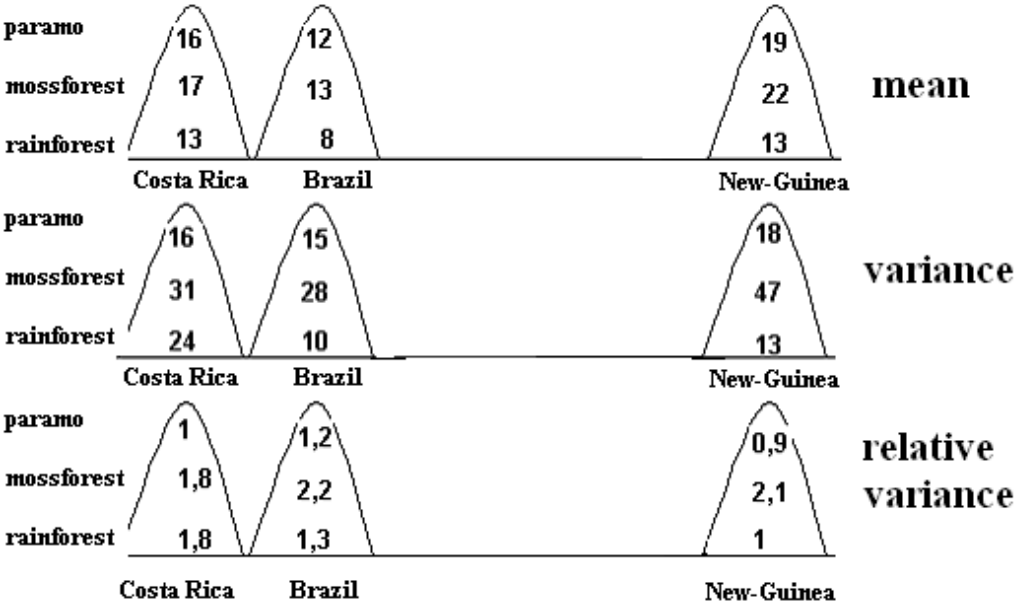

Figure 2. *Average genera numbers and variance of families belonging to genera of individual habitats*

Zoogeographical indicators

For the purpose of zoogeographical analysis, we used the information of worldwide prevalence of representatives of individual genera, based on the data of *Table 2.* We introduced the method of "simple voting" where the occurring genera give one vote on the zoogeographical regions where at least one of their representatives lives. Summing up the zoogeographical votes, the obtained score characterizes the "faunagenetical affinity" of the habitat to zoogeographical regions. Sums of obtained votes are shown in *Figure 3*. It can be read from the figure that Neotropis and Notogaea votes were obviously highest on their own sites, but it is interesting to observe that this value is always highest in the rainforest and it gradually decreases by ascending towards the top of the mountain. This means that in mossforests and paramos the influence of other regions (and cosmopolites) is higher. In Neotropis this phenomenon shows in the growing role of the Holarctic fauna, while in Notogaea the role of Antarctic fauna

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grows. The effects of Orienthalis and Ethiopis do not differ significantly from each other, which may be surprising in respect of the New-Guinean area. The effect of Orienthalis is the strongest always in rainforests, while the influence of Antarctic is most significant in paramos.

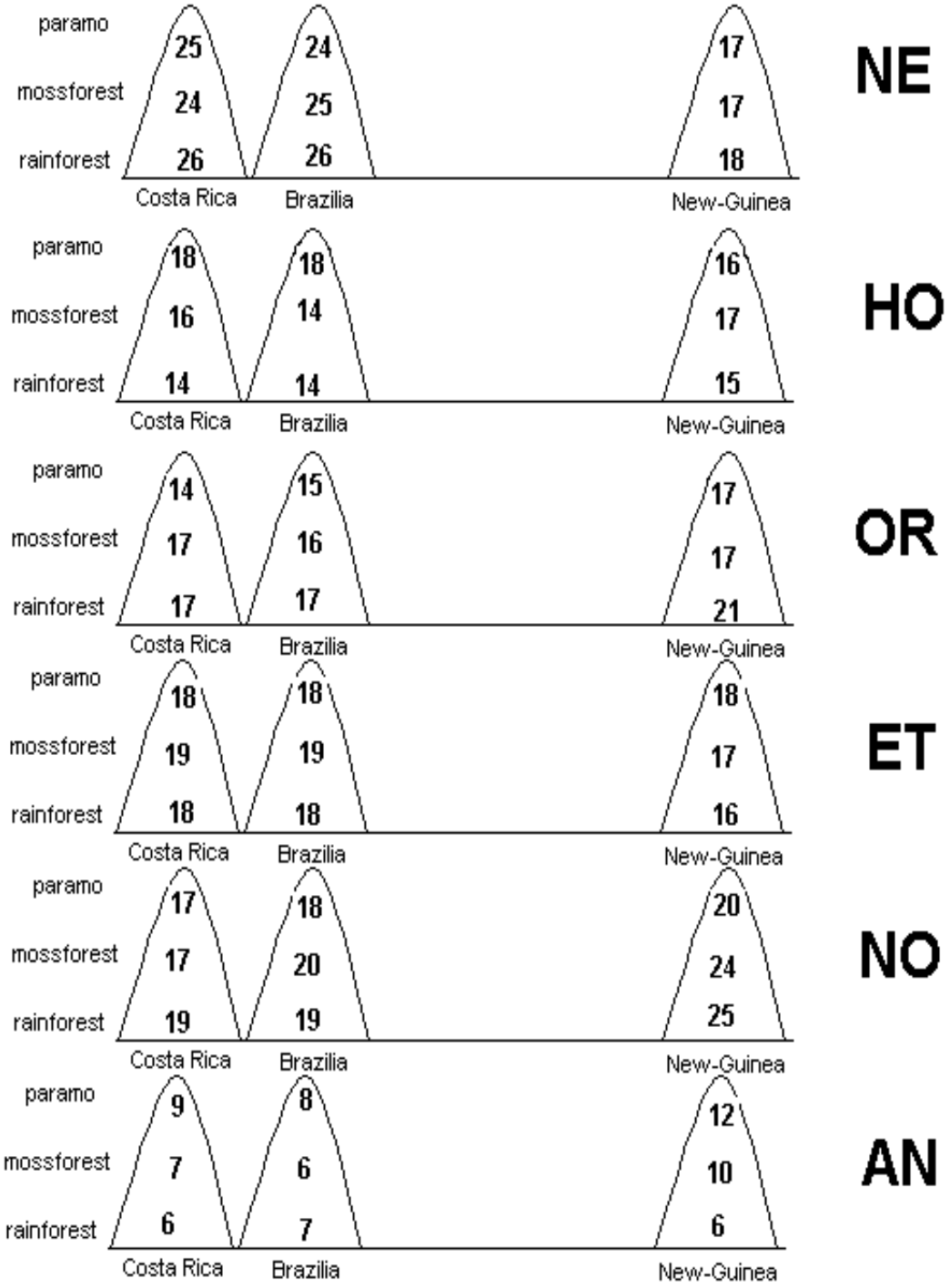

Figure 3. *Sum of votes of individual sites for the world's zoogeographical regions, based on the occurrence of representatives living there*

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In respect of Antarctis (AN) it can be stated that with its significantly lower sum of votes it sharply distinguishes from data characteristic to other continents. Highest values can be found in New-Guinean paramos and mossforests, and high values can also be found in the paramo belt of the American continent. Low values can be observed in the rainforest and mossforest habitations of the American continent. As for Neotropis (NE), the highest values can be found in the rainforests of the American continent, and the lowest ones in the New-Guinean paramo and mossforest habitats. Based on votes of Notogaea (NO) the lowest values can be observed in the higher regions of Costa Rica. Votes of Ethiopis (ET) and Orienthalis (OR) regions seem similar, but it is clearly visible that the votes of Ethiopis are generally higher in all areas except the New-Guinean rainforest, which shows paramount Oriental influence, however the effect of Ethiopis is lower in this region. The lowest sums of votes of Orienthalis (OR) region can be found in the Costa Rican and Brazilian paramos. The highest values of the Holarctis (HO) region can be observed in the paramos of the American continent, while the lowest influence can be experienced in the rainforests.

Heterogeneity indicators

We consider as an important question of indication that in what extent the general structure of soil samples collected from different habitats differ from each other, which describes how heterogeneous coenology the site in question has generically. As a measure of heterogeneity, we chose the average pairwise difference of individual samples of identical sites, which had been evaluated by using two indicators with largely different biometrical characteristics: PHI coefficient and Jaccard function (*Figure 4*). According to the examinations, paramos were always the most heterogeneous, and mossforests were always found to be the most homogeneous. Heterogeneity of the rainforests stood always closer to the mossforests. Highest heterogeneity was shown by the New-Guinean paramo, while the lowest by Brazilian mossforest according to both distance functions.

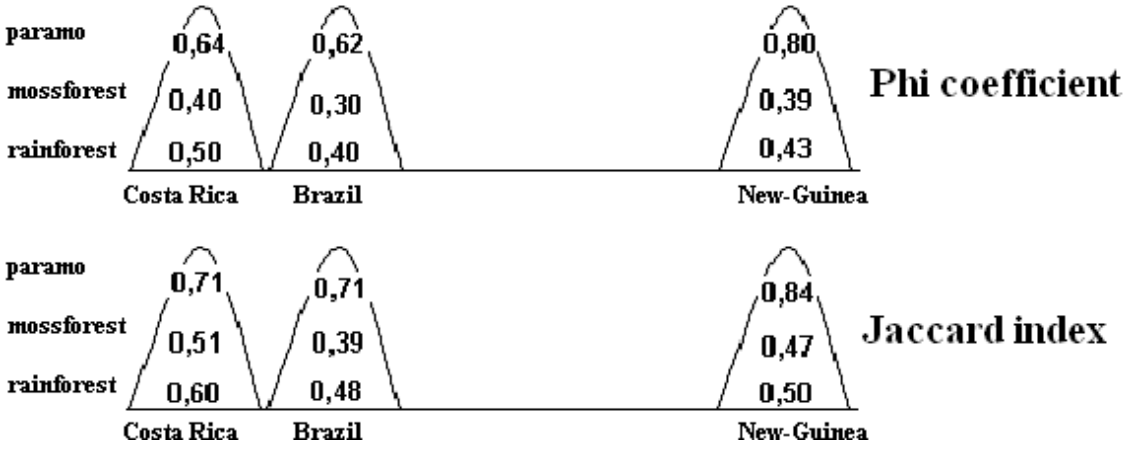

Figure 4. *Generic heterogeneity of individual habitats based on the average difference of individual sample pairs*

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Coenological similarity patterns as indicators

Coenological similarity patterns can be analysed on multiple spatial scales (scale levels). We also analysed the similarity patterns of generic lists of different sites by NMDS with Euclidean distance, and hierarchical cluster-analysis. The two analytical results are shown projected onto each other (*Figure 5*). The multivariate similarity pattern of habitats' Oribatid community gave the expected picture. It can be stated that differences originating from the habitats and continents can also be recognized in the similarity patterns of the generic lists of the examined habitats. However, it can seem surprising that despite the vast geographical distances the pattern generating role of habitats does not disappear, it seems perhaps even more important. In accordance with the real ecological conditions, mossforest plays a transitional role between rainforest and paramo. However, mossforests are the most similar to each other and they are positioned in the middle of the similarity pattern, while the rest of the sites are separated radially. It is clearly visible that Brazilian sites are much more similar to each other than the Costa-Ricans.

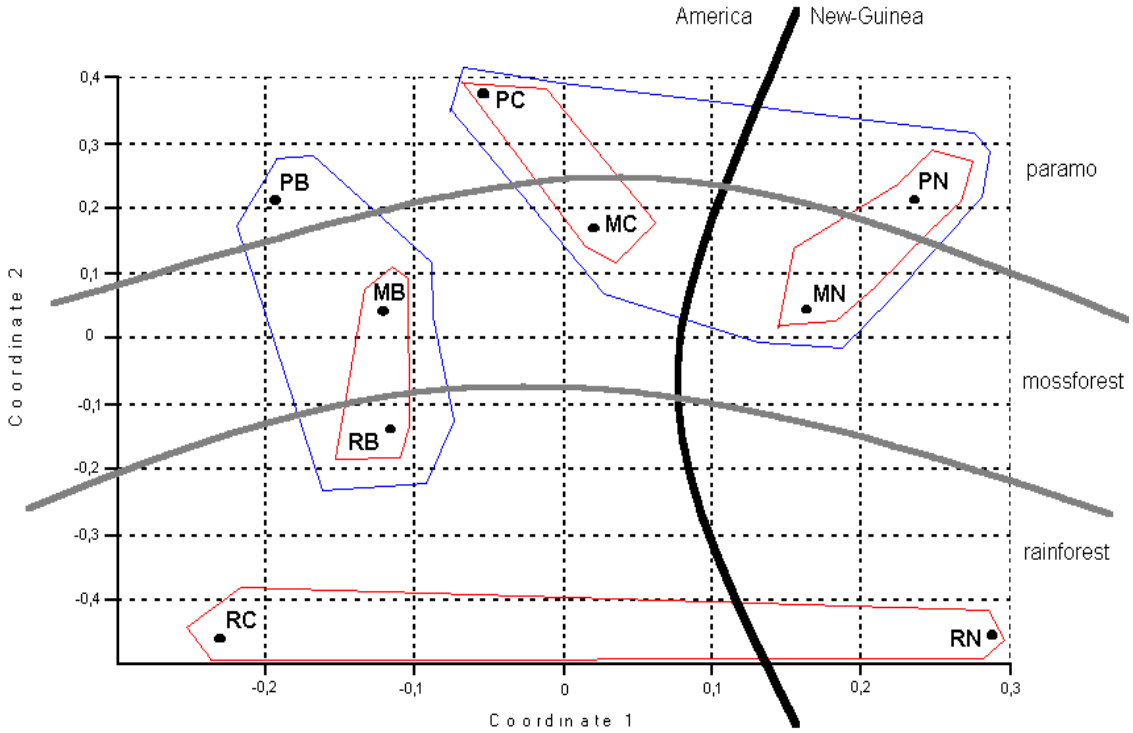

Figure 5: *Similarity pattern of the examined sites in an NMDS ordination with the projection of the hierarchical cluster analysis results, applying Euclidean distance.*

Comparison of the sites can be fine-tuned if we also examine the similarity pattern of the individual soil samples considering every sample as different objects independent from the sites. We analysed this similarity pattern also by applying NMDS and Euclidean distance (*Figure 6*). Analysing the collective similarity pattern of every individual sample it can be stated that the groupings of elementary samples reflect their relations to the sites. This justifies the methodological decision by which sites are considered the basic objects of the examination. It can be stated furthermore, that habitat-type is unambiguously more significant pattern-generating factor than

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geographical attribution. Rainforests, mossforests and paramos lying thousands of kilometers from each other are more similar than sites of other habitats at only a few kilometers distance. Following the results of these pattern analyses, exact examination of the observed phenomena with a regression model seems to be practical.

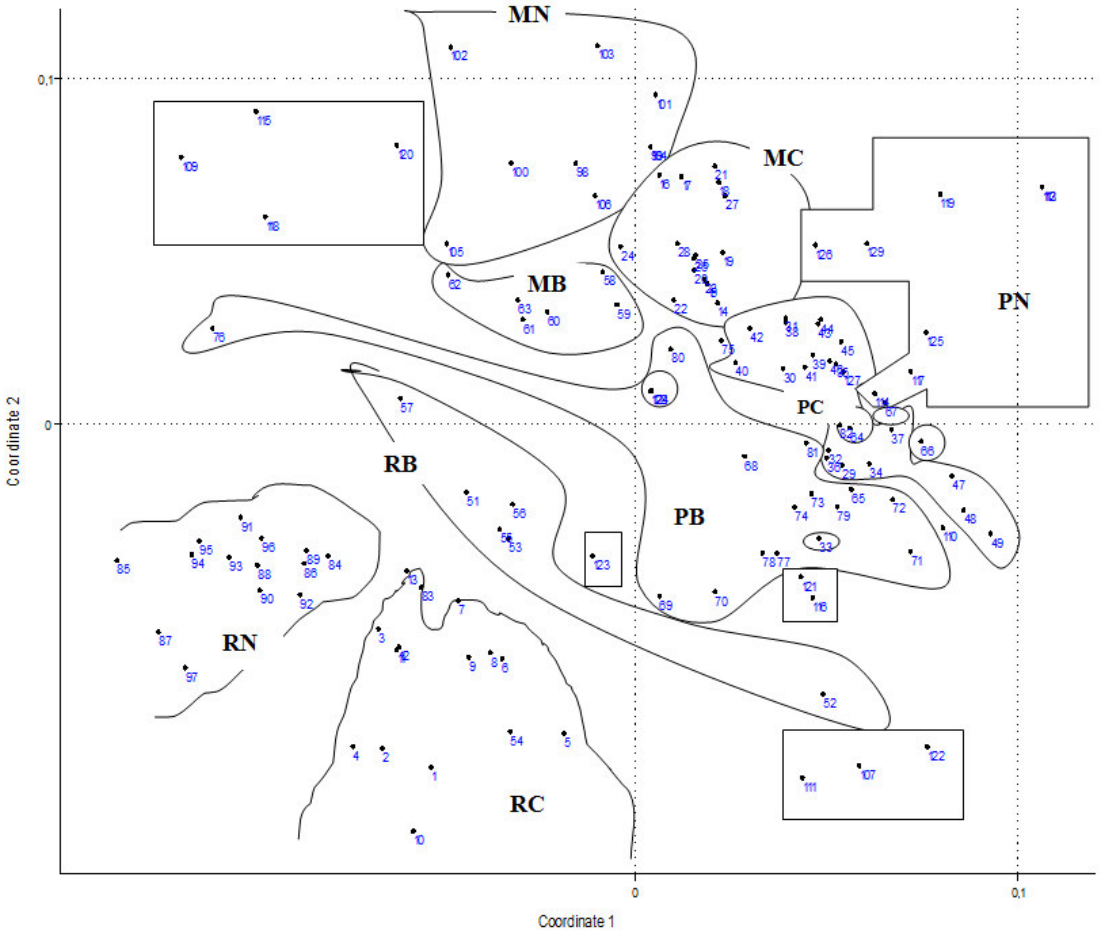

***Figure 6**: Similarity pattern of elementary samples in an NMDS ordination by applying Euclidean distance.*

With the help of the tools of regression modelling we tried to describe what kind of factors and in what extent explain the occurrence of Oribatid genera.

For the purpose of exact statistical examination we classified the 82 individual soil samples into groups by habitats, habitat-types and continents. We distinguished samples originating from the identical site, identical habitat (habitat-type) and identical geographical unit. Samples from different geographical units were allocated into two groups depending whether they were collected from identical or different habitat-types. For the purpose of identifying the pattern generating factors we created criteria for the statistical evaluation based on this classification as follows:

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Criterion	Number of samples
In-site	9
Inside habitat	9
Inside continent	9
on other continent	12
on other continent from neighbouring habitat	6
Total	**45**

During the statistical analysis we based our calculations on 45 distance values which were created by the following method: the 82 samples contained 111 Oribatid genera. Consequently we characterized every sample with a column vector of 111 elements. The elements of these vectors were created as follows:

- 1: on those places, where the given genus is present in the sample
- 0: on those places where the given genus is not present in the sample.

With the help of the binary data created this way, we intended to examine how 'similar' two samples are. There are many indices in biometry for similarity (Podani 1997), since the task of biological examinations is often the numerical measurement of an intuitively defined similarity. In case of binary data sets, there are many possibilities to measure similarity. For the analysis we used the product moment correlation (PHI) coefficient. This coefficient was created with the help of the Syntax program, for every sample. This resulted in a distance matrix, where the elements of the matrix are the values of PHI coefficients calculated between the two samples.

During sample collection, researchers have taken many samples from a habitat, thus one habitat is characterized by many samples. In order to filter out the sample-specific effects, we took random samples consisting of 6 elements from the calculated distances for each examined criterion. The criterion has been characterized by the average and variance of these 6 values:

Table 3. *Statistical characteristics of criteria.*

Criteria	Number	Average	Variance	Relative variance
in-site	9	0.612	0.011	1.82%
in the same habitat	9	0.864	0.008	0.87%
in the same geographical unit	9	0.894	0.003	0.38%
on other geographical unit	12	0.976	0.004	0.44%
on other geographical unit. in a neighbouring habitat	6	1.002	0.003	0.28%
Total	**45**	**0.883**	**0.006**	**0.63%**

Further analysis consisted of two parts. First, with the help of graphical analysis (*Figures 7 and 8*) we set the starting hypotheses, and then numerically measured the suspected effects with multivariate regression and examined the significance of the obtained conclusions.

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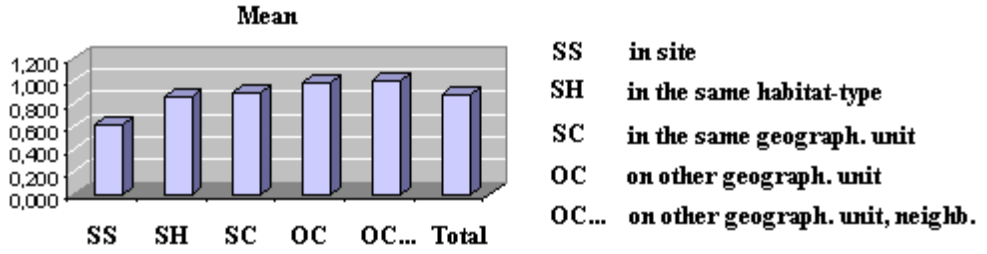

Figure 7. *Average distance/similarity of criteria.*

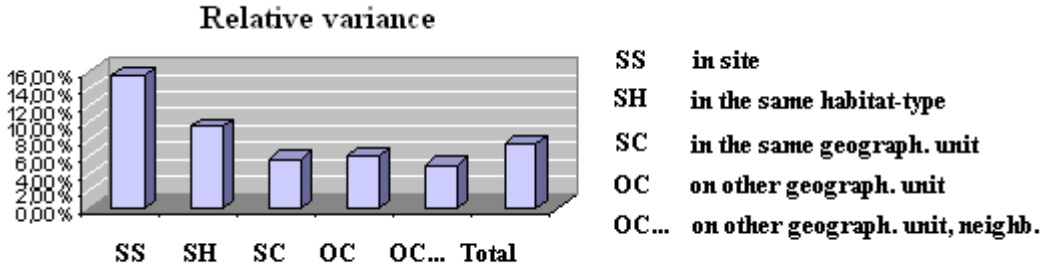

Figure 8. *Relative variance of distance/similarity of criteria.*

Based on *Figures 7 and 8*, it seems that the difference between in-site samples is significantly less than that of between other samples. On one hand, this affirms sample collection and, on the other hand it reaffirms the decision of the researchers selecting genus as the basic unit of analysis. The variance of in-site samples is significantly higher than that in other cases. Based on the figures the null-hypothesis can be supposed that the difference is significantly less in-site. Between geographical units, the distance of samples seems significantly higher than in the case of samples on identical geographical units. The question emerges whether the geographical unit or the habitat type defines better the distance between the samples. Are genera inside a habitat significantly more similar than in case of samples collected from the same geographical unit but from different habitats? We tested these null-hypotheses by using uni- and multivariate statistical tests.

First, we are going to interprete the result of the univariate model and then improve the model by implying more variables.

We conducted the test by using the Eviews statistical program. During the examination, we checked by using the t-test whether the given variable is significant or not. The null-hypothesis of the test was that the coefficient belonging to a given variable was zero. Instead of t values, we used p (marginal significance coefficient) values in the tables, which show the probability of rejecting the null-hypothesis. The advantage of using p values is that there’s no need to work with a pre-set level of significance, instead, the probability of beta error can be reduced according to the estimation.

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First, we examined if there's significant difference among the average distances between the samples grouped by different criteria. We tried to check the null-hypothesis by using linear regression. In case of every regression, the null-hypothesis was that the distance of the sample grouped by a given criterion differed significantly from the average distance characteristic to the samples. Hypotheses could be tested by using dummy-variables. For example:

site = 1, in case of sample taken from the identical site

site = 0, in case of sample taken from a different site.

The average distance of samples is marked by: distance. In this case, the estimating equation:

(1.) $dis\tan ce = \beta_0 + B_1 * site$

if, for example the t-statistic belonging to β_1 is significant, then the location of sample collection explains the distance. By implementing similar dummy-variables, the estimation to characterize continents and habitats can be made.

Table 4. *Dummy variables defined for the examination*

Criterion	**Dummy**
in-site	SITE
inside the habitat	HABIT
inside the geographical unit	CONTINENT
on other geographical unit	NOCONTINENT
other geographical unit on a neighbouring habitat	NOCONTINENT2
Calculated similarity of the samples:	**DISTANCES**

The results of the estimation are summarized in *Table 5*. C(1) marks the constant in every case.

Table 5. *Result of univariate linear regression estimations*

Dummy variable	**Estimating equation**	**C(1)**	**C(2)**	**Significance level**	**R-square**
Site	DISTANCES = C(1) + C(2)*SITE	0.94	-0.32	0.00	0.60
Habit	DISTANCES = C(1) + C(2)*SITE			0.68	
Continent	DISTANCES = C(1) + C(2)*CONTINENT			0.55	
Nocontinent	DISTANCES = C(1) + C(2)*NOCONTINENT	0.85	0.13	0.01	0.14
nocontinent_2	DISTANCES = C(1) + C(2)*NOCONTINENT2	0.86	0.14	0.04	0.10

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Based on the results above, we can clearly see that the distance between samples taken from identical sites is significantly less than that in the case of other samples. This means that the examined genera statistically characterize a given habitat. Otherwise: when comparing two soil samples it can be determined whether they are from the same site or not by the examination of Oribatid genera. Thus the examined animals can be used for indication purposes.

Further results show that the distance of samples between geographical units is greater in average than that in the case of identical geographical units. However we can't be sure that this result is caused because the samples collected from the same site belong to the identical geographical unit, and these samples significantly reduce the average difference. In a similar way, it can also be supposed that the distance between the samples belonging to the same habitat is not significant because the distance is biased by the effect of samples from the same site. This is why we re-estimated the regressions in such a way that the site dummy would have been included in every regression. The results of the estimation are summarized in *Table 6*:

Table 6. *Result of bivariate linear regression estimates*

Dummy variable	Estimating equation	C(1)	C(2)	Significance level	C(3)	Sign. level	R-square
Habit	DISTANCES = C(1) + C(2)*SITE + C(3)*HABIT	0,960	-0,348	0,000	-0,096	0,010	0,665
continent	DISTANCES = C(1) + C(2)*SITE + C(3)*CONTINENT	0,944	-0,332	0,000		0,399	0,611
nocontinent	DISTANCES = C(1) + C(2)*SITE + C(3)*NOCONTINENT	0,916	-0,304	0,000	0,060	0,089	0,632
nocontinent 2	DISTANCES = C(1) + C(2)*SITE + C(3)*NOCONTINENT2	0,923	-0,310	0,000	0,079	0,076	0,634

Based on *Table 6* it can be stated that the site dummy is also significant at 1 % level in every case. This affirms the result that the genus-level classification of Oribatid genera is sufficient, and furthermore, their distribution characterizes a soil sample in a statistically significant manner. So these genera can be well used for biological indication.

Among further dummy variables, the variable characterizing the habitat is significant even at 1 % level. This means that two samples collected from the same habitat are more similar than two other kinds of samples. Thus, the habitat characterizes the distribution of the genera more significantly than the geographical unit the sample originates from. In case of different geographical units, the difference between the samples is only significant at 10 % level of significance. This is why the acceptance of this hypothesis greatly enhances the probability of beta error.

Following the aforementioned methodology, proceed from the simple towards the complicated and repeat the estimation with the joint application of variables characterizing habitat and geographical unit (*Table 7*).

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Table 7. *Result of regression estimates using three variables*

Dummy	Estimating equation	Coefficient	Significance level	R-square
C	DISTANCES = C(1) + C(2)*SITE + C(3)*HABIT + C(4)*CONTINENT	0.984	0.000	0.697
SITE		-0.372	0.000	
HABIT		-0.121	0.002	
CONTINENT		-0.073	0.052	

Table 7 shows that all variables in the model are significant at 6 % level. Thus, by accepting the model specification, the probability of beta error is limited. The average value of the difference of two random samples is **0.984**. If these samples are collected from the same continent, this difference is by **0.073** lower, and if the samples are from the same habitat, this difference is by **0.121** lower than the average value. An interesting result is that the distribution of genera is heavier influenced by the habitat type than the geographical unit (the size of which, in our case is practically of continent scale). If samples are from the same site, the average distance is by **0.0372** smaller than the average value. Model explains 70 % of the deviation from the average.

To check the specification of the final model, we examined the distribution of the error components. An important condition of the statistical analyses grounding the final result is the normal distribution of error components. We checked the normal distribution of error components with two kinds of methods. First, we displayed the distribution graphically, and then checked the distribution of error component with the Jarque-Bera test. This test compares the difference between **skewness** and **kurtosis** with that of normal distribution. The null-hypothesis in this case was that the distribution of the samples is normal. The displayed probability shows the level of significance at which we could reject the null-hypothesis.

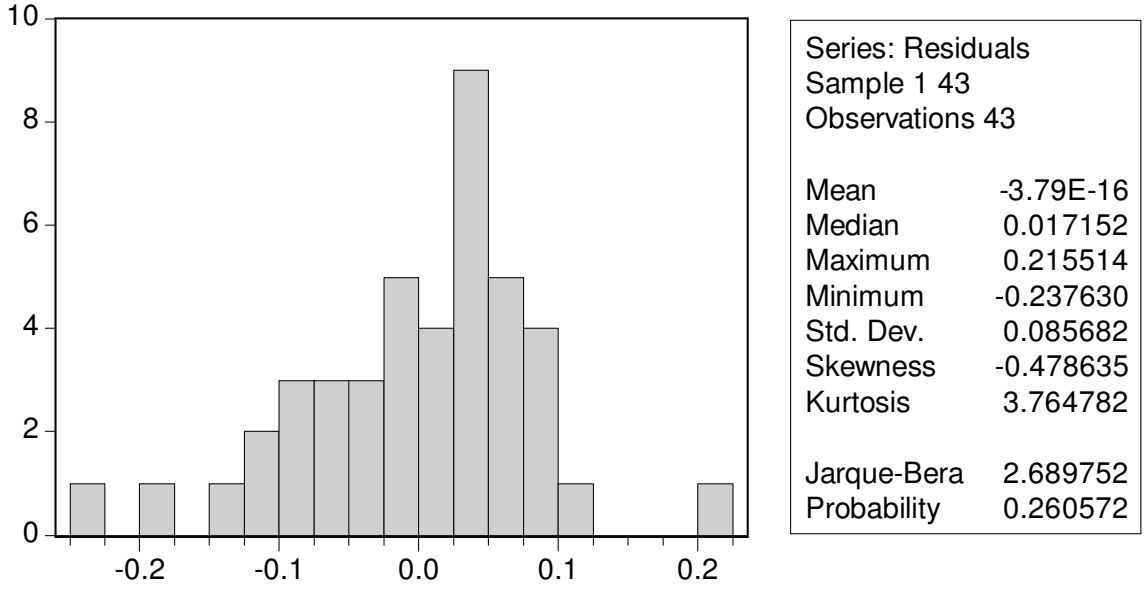

Figure 9*: Examination of error coefficient of the final model.*

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As *Figure 9* shows, the distribution of error coefficient converges well to normal. We can not reject the null-hypothesis of the normal distribution even at 10 % level. Thus we accept the model in its final form.

Theses

1. We pointed out that the average species number of those genera where the present Oribatids belong to, can be properly used for the characterization of habitats Concerning Costa Rica, Brazil and New-Guinea, we found that this indicator shows a continuous growth advancing upwards from the tropical rainforests (towards the belt of mossforests and paramo vegetation). Examining the empirical variance of this indicator, it can be stated that the least variance can be found at the level of rainforest in every case. This means that members of genera with greater species numbers live at higher elevations.

2. The average genus number of families of Oribatid genera present at individual habitats also proved to be a useful indicator. We pointed out that in case of all the three examined locations, representatives of families with the greatest genus numbers live in the mossforest, and representatives of families with the lowest genus numbers live in the rainforest. In this respect, paramo always stood closer to mossforest. Furthermore, we pointed out that the relative and empirical variation of this indicator is the greatest in the mossforest.

3. Concerning zoogeographical indication, we pointed out that the method of "simple voting" can result in well-interpretable indicators in respect of the generic relativity of individual habitats and main zoogeographical regions. Votes of Neotropis and Notogaea were highest in their own sites of course, though interesting that this value is always the highest in the rainforest and gradually decreases advancing upwards the hill. This means that the influence of other regions (and cosmopolites) is greater in the mossforests and paramos. This phenomenon manifests in Neotropis in the growing influence of Holarctic fauna, and the growing influence of Antarctic fauna in the Notogaea. The effects of Orienthalis and Ethiopis do not differ significantly, which can be surprising in respect of the New-Guinean location. However, the effect of Orienthalis is always the strongest in rainforests, while paramo has the strongest Antarctic influence. But the small difference between the votes calls for the usefulness of later inclusion of new taxa.

4. Examining the proportion of species below 20 % and 40 % constancy, we pointed out that these indicators are always the highest in paramos.

5. We pointed out that the information on the extent of heterogeneity of the genus composition of the soil samples of an individual habitat can be well used in the characterization of the habitat. Evaluating by PHI coefficient and Jaccard function the average pairwise dissimilarity of the individual samples from identical sites as the measure of heterogeneity, we always found the paramos to be the most heterogeneous, and always the mossforests to be the most

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homogeneous. The heterogeneity of the rainforests always stood closer to that of mossforests'.

6. We pointed out that dissimilarities of habitats caused even by their type and also by the continent they originate from can be recognized in the similarity pattern of genus lists of the examined habitats. But if we analyze the overall similarity pattern of all the individual samples, it is quite conspicuous that the type of habitat is a much more significant pattern-generating factor than the geographical location. Rainforest, mossforests and paramos located many thousand kilometers from each other are more similar to each other than sites of other kind of habitats in only a few kilometers away. This statement is even statistically verified by the multivariate linear regression fitted to our data.

Discussion and conclusion

The most important result of our case study is that the list of Oribatid genera as a coenological indicator, primarily characterizes the present ecological effects of the habitat and its climatically determined type of vegetation; and represents the effect of zoocoenological past in a much lesser extent. Thus, it can be concluded that a meritable scientific faunagenetical analysis should not be based upon geohistorical, but climatological grounds. This is why the ecological indication based on Oribatid genus lists provides unique possibilities for the purpose of climate change research.

It became obvious that if we had chosen species and not genera as the basis of our examination, we would not get results that were interpretable from bioindicational aspect. This is because the majority of the described species would have appeared only as local specifica, and they do not provide any meritable information on similarity patterns – unless we are thinking in a very small grade of space. Species-level indication studies would be only rational in the case if we would deal only with cosmopolite species, but their number and detectability would not be sufficient for the majority of examinations for practical tasks. Thus, our important conclusion is that under current circumstances, the recommended taxonomical unit for indicational ecological studies can only be the genus. This statement is also important because many authors (primarily species describing taxonomists) consider a serious problem of indicational research that a number of researchers classify individuals only to genus level (Gulvik 2007). However, based on the work of Caruso (2006), we know that human contamination, intervention and disturbance can be better detected if we examine larger taxonomical units. It is also clear that rapid changes can not be detected on species-, but on community level. From the same work, it is also known that the species data of Oribatid mites can be raised to genus- or family level, and this does not cause loss of data or sensitivity by multivariate methods. According to Osler (1999), it is possible that habitat preference is determined rather at family level. Furthermore it is also known that there is strong relation between species-level and higher taxon level diversity indicators.

At the same time, the standardization and quantification of current Oribatid-collection methods would be obviously necessary for the development of the bioindicational methodology in order to make the data from different authors comparable. The need for this is emphasized more and more in literature (Gulvik 2007). A criteria-system of classifying the collected individuals into genera (or into other optional morphological groups) can be considered as a part of the standardized method.

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The current identificational and taxonomical practice in oribatodology is almost completely unsuitable for the purposes of biological indication research. Till such methods are unavailable, case studies can be conducted only if the person doing field sample collection and taxonomical processing is identical, and furthermore, this is only true if the coenological matrices have been created with the greatest care.

During our examinations, we pointed out that by advancing vertically upwards in the tropical high mountains (from rainforests towards the paramo vegetation), the average species number of genera present, and the extent of Holarctic and/or Antarctic relations of present genera grow, which can be well interpreted with the conception system of geographical analogy based on climatic similarities. This indication adequately supports former studies on the role of vegetational similarities and on genera as taxonomical units suitable for indication. Many authors (Andrew 2003, Melamud 2007) state that advancing upwards on the mountains, elevation above sea level and exposure have significant influence on the diversity of Oribatids. Besides, it is known that Oribatid diversity grows from the Boreal region towards warmer climates, but it does not grow further towards the tropics (Maraun 2007).

Furthermore, we pointed out that from the aspect of similarity of individual samples, among the examined habitat types the mossforest is the most homogeneous habitat, and paramo is the most heterogeneous. This latter phenomenon can be well interpreted if we consider the uniform moss cover prevalent in the mossforest, and the role of stable microclimate created by the moss cover. In the paramo, the observed proportion of species with low constancy level and also the heterogeneity of habitats (tussocks with different size) are high, and the role of the resulting microclimatic variability is obvious.

Acknowledgements. The late academician prof. János Balogh suggested and oversaw the beginnings of the research presented in this paper, and his scientific legacy was indispensible in completing it. We express our acknowledgement to his family.

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BOOK REVIEW: ANTHROPOGENIC GEOMORPHOLOGY

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University of Debrecen, 2006.
Kossuth University Press, Debrecen, 322 p. (ISBN 963 472 979 7)

In the past few decades interest in the environment has reached a peak as popular opinion has become aware of the extent of the human impact on natural systems. A proliferation of degrees has followed this wave of 'environmentalism', their focus has been on natural areas and the damage caused by human impacts. **Environmental geomorphology** is a special interaction of humans with the geographical environment which includes not only the physical constituents of the Earth, but also the surface of the Earth, its landforms and in particular the processes which operate to change it through time. Since the 1970s in the research of the physical environment two, frequently intertwining trends are prominent. One of them investigates the changes in the natural environment induced by human economic intervention (which are often undesirable) along with their counter-effects. The other aims at the quantitative and qualitative survey of the resources and potentials of the physical environment and the evaluation of also regionally varying geographical potentials. Researchers reviewing the geomorphological literature of the last 40 years will gain the impression that the perception of Man as a geomorphological agent is a fairly recent development. **Anthropogenic geomorphology** is a new approach and practice to investigate our physical environment, because in the eighties the more and more urgent demands from society against geography - ever more manifest due to the scientific-technical revolution - underlined the tasks to promote efficiently the rational utilization of natural resources and potentials, to achieve an environmental management satisfying social requirements and opportunities. At the same time, anthropogenic geomorphology is a new challenge for geomorphologists, since environmental problems have an effect on several branches of science. Anthropogenic geomorphology studies the huge – and more and more increasing – number of landform associations of extreme variety depending on the given way and aim of their creation, which have been made by the human activity. The discipline also studies the surface changes induced by these forms; moreover, predicts the consequences of disturbance of the natural equilibrium, and makes recommendations for preventing damages. Therefore, anthropogenic geomorphology can be also regarded as an applied discipline, which helps to solve both social-economic as well as environmental and natural protection problems.

The editors and authors of the new Hungarian text-book on anthropogenic geomorphology think that mankind must be regarded directly as a geomorphological agent, for it has increasingly altered the conditions of denudation and aggradation of the Earth's surface, and it has been becoming the main landscape sculpturing factor. We regard and teach anthropogenic geomorphology as an activity system, therefore, we believe in the equality ranks among the various fields of science in environmental protection and we assign an important part to anthropogenic geomorphology in the structure of our education. The organisation of the book follows this concept. After a general introduction of aims and scope of this discipline, the individual chapters focus on the different sectors of the human activity. The final chapter intends to give a qualitative and quantitative summary of the human impact on the Earth's surface.

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