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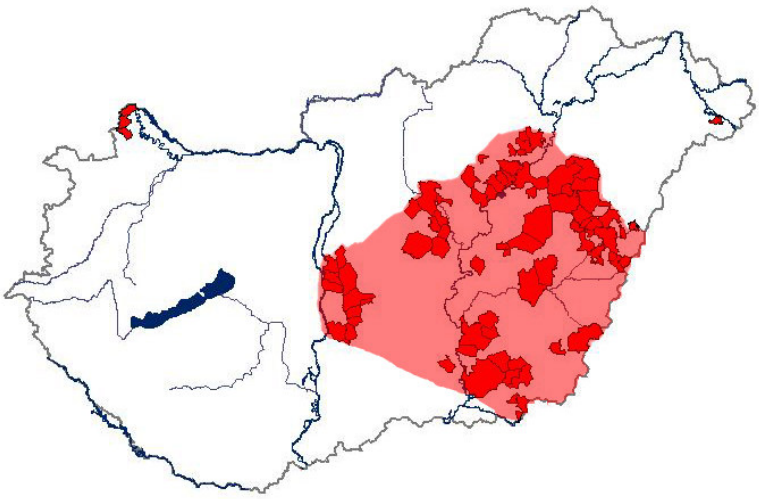

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MONITORING MULTISPECIES INTERACTIONS: A CASE STUDY OF 16 MAIN TREE SPECIES ALONG THE NORTHEAST CHINA TRANSECT

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Abstract. Viability of single species can be understood only in the context of ecological interactions with the other species and the environment. Monitoring multispecies interactions and their environment is critical for analysis of community dynamics, multispecies habitat conservation plans and for adaptive ecosystem management programs. In this study, three simple methods (scaling exponent of Taylor's power law, resource exploitation competition and interspecies mean crowding coefficients) based on abundance were applied to estimate multiple tree species interactions along the Northeast China Transect. This transect was identified as a middle-latitude transect for terrestrial ecosystem studies by Global Change and Terrestrial Ecosystem Program of IGBP. Our analyses show the differences and similarities of these three methods because each one emphasizes different aspects of multispecies interaction measurement. The combined use of these three methods can provide an easy and simple way to estimate multispecies interactions based on abundance of each tree species and their change under environmental change. The suggested approach could help identify indicator species for monitoring, improve population viability analysis, and set priorities species conservation.
Keywords. *Interspecies mean crowding coefficients, multispecies habitat conservation plans, Northeast China Transect, resource exploitation competition, Taylor's power law*

Introduction

The single species approach to monitoring is considered inefficient and ineffective [2, 5, 21, 23, 24]. The main issues confronting the species-by-species plan [23] are (i) entire communities rather than single species need to be the focus of conservation efforts, because species may rely on each other; (ii) information about vulnerable communities and their constituent species may be limited due to underlying complicated processes; and (iii) species always interact with each other in complicated ways. Several competitors may affect one species; the complex food webs and environmental fluctuations at a large area have different implications for different species.

Multispecies habitat conservation plans (MHCPs) have emerged and are designed to conserve the biodiversity of a region by ensuring that a representative set of species are protected and also minimize conflicts from species-by-species approaches [2, 24]. The critical requirement of these plans is monitoring site specific multispecies interactions and the interaction with the environment, which mean competition for space and resources or impact from other species and environment. Such information can also be useful for adaptive management programs. Most studies on multispecies interactions concentrate on using sophisticated models to estimate competition coefficients between species under given conditions of environmental change [10, 18]. However, building those models is difficult because it requires sufficiently detailed information and

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estimation of some parameters that are hard to measure [12, 25]. Simple and practical ways to monitor the interactions of multiple species by easily obtained and less long term time series data are much more desirable for local or regional MHCPs.

Multispecies interactions directly or indirectly impact the abundance of each species. Classical theory asserts that each species is unique in its resource demand and the resulting variation in interspecific interactions that determines biodiversity and relative abundance in a community [7]. It would be useful to be able to detect multispecies interactions from abundance of each species. These interactions could be easily monitored by data series in time or space. Currently there are three simple methods to estimate multispecies interactions by abundance. The scaling exponent of Taylor's power law was used to describe interactions from other species and environments [17]. Lloyd (1967) measured interspecies mean crowding by species abundance [20]. Measurement of mean crowding represents the extent of spatial crowding. The species resource exploitation competition coefficient was used to estimate interaction coefficient in using resources [19]. Here these three methods based on abundance analysis for monitoring changes in interactions among multiple species were compared for tree species along the Northeast China Transect (NECT). NECT was one of middle-latitude transect for terrestrial ecosystem studies by Global Change and Terrestrial Ecosystem (GCTE) of IGBP [13]. Because this transect is parallel with latitude, its vegetation change is driven mainly by moisture. The annual precipitation is as high as 800 mm in the east and only 100 mm in the west along this transect. Monitoring the change in interaction among tree species under precipitation gradients would be helpful to study these species dynamics at a large region under environmental change. Therefore, the aims of this research are to (1) compare the results of these three methods; and (2) find a simple method or synthesize a new one based on easily measured indicators (such as abundance) and less long term time series data, to detect species viability, multispecies interactions and their changes under a changing environment.

Materials and methods

Study area

Our study area was approximately from longitude 125ºE to 130ºE along latitude 43.55ºN. The total length was about 400 km. The data set was selected from 100 permanent plots (each plot 30×30 m^2) sampled every 4 km in 1986 and 1994. The soil type in this area is dark brown soil. Every tree with a diameter at breast height larger than 2 cm was recorded. Detailed information about the study area can be found in [4, 6].

Statistical methods

There are many studies about species interactions, however, based on simple and practical criteria, the following three methods for estimating species interactions based on abundance analysis were used in this study:

(1) Scaling exponent of Taylor's power law: Taylor's power law originally described the species-specific relationship between the temporal or spatial variance of populations and their mean abundances [27, 28]. The negative interactions from the other species and environment in a community can produce a scaling exponent of Taylor's power law of less than 2, and that the scaling exponent decreases with increasing strength of

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interspecific competition [17]. Kendal (2002) explained Taylor's power law by an exponential dispersion model and tested it by spatial aggregation of the Colorado potato beetle [16]. In this study, the scaling exponents of the log values of mean abundance of each tree species and variance in 1986 and 1994 were estimated for the entire area and for different lengths along NECT. The results of each tree species were compared with each other and the null hypothesis of a slope of 2.0 for no interspecific interaction. Mathematically, the logistic distribution which corresponds to the logistic equation yields a scaling exponent of exactly 2. The slope may increase to 2.5 if a population is divided among heterogeneous areas with dynamically autonomous subpopulations and migration among them [1]. A reduced major axis (RMA) of regression analysis Model Type II was used to determine scaling exponents for different species in different areas. The details of statistical procedures can be found in [26].

(2) Species resource exploitation competition coefficients were estimated as the following [8, 19].

$$\alpha_{ij} = \frac{\sum_h p_{ih} p_{jh}}{\sum_h p_{ih}^2} \qquad \text{(Eq.1)}$$

where p_{ih} is the fraction of individuals found in location h that are of species i; p_{jh} is the fraction of individuals of other species; α_{ij} is the interaction coefficient of species i from all other species. Some workers have used the above formula or modifications thereof to measure niche overlap [11]. This formula was also used to estimate competition coefficients of ants [9]. A test of this measurement was conducted for birds in southern California and on Santa Cruz Island [29].

(3) Interspecies mean crowding was estimated by [14, 20]:

$$m_{XY} = \frac{\sum_{j=1}^{h} x_{Xj} x_{Yj}}{\sum_{j=1}^{h} x_{Xj}} \qquad \text{(Eq.2)}$$

where m_{XY} is the mean crowding on species X from species Y, and x_{Xj} and x_{Yj} are the abundances of species X and Y in the jth quadrat, respectively. The m_{XY} was used to analyze the spatial association between two species [14]. The relationship between the mean crowding coefficient and the mean abundance was used to discuss a variety of biological distributions [15]. In this study, the mean crowding interaction on each species from all other species at different locations (with increase of distances) was estimated.

All three metrics can measure multispecies interactions, but their results may be different due to their different aims. Comparing the results of these metrics provides a potential method for monitoring multispecies interaction for the purpose of monitoring, conservation and forest management.

Data compilation

Abundance or the number of individuals of each tree species in each plot along NECT was recorded. Abundance of each species at different spatial locations along NECT was aggregated with the distance of every 50 km from the beginning. The fraction of individuals of species i in each location or along different lengths of NECT

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was calculated as the percentage of abundance of species *i* of the total abundance of all species. log values of mean abundance of each tree species and variance in 1986 and 1994 were calculated for the entire study area and for each 50 km along NECT. Then, RMA of regression analysis Model Type II was used to determine scaling exponents between log (mean abundance) and log (variance of abundance) for different species in different areas.

Results

The overall interaction coefficient of each species at NECT

The overall interaction of each species from all the others and environment can be estimated by the scaling exponent of mean abundance and variance on NECT in 1986 and 1994 (*Table 1*). In 1986 the scaling exponents of *F. mandshurica* and *L. olgensis* were not significantly different from 2.0, and this means that these two species had almost no interaction with other tree species. In 1994 *P. amurense*, *F. mandshurica* had little interspecific interaction. The interaction strength of *U. pumila*, *P. koraiensis*, *A. mono*, *L. olgensis* and all others increased significantly from 1986 to 1994, respectively. For the others, the interaction did not change significantly.

Table 1. *The overall multispecies interaction strength of each tree species along NECT in 1986 and 1994*

Tree species	Short names	Scaling exponent of Taylor's power law		Species competition coefficient		Interspecies means crowding	
		1986	1994	1986	1994	1986	1994
Betula platyphylla	Bp	0.7239	0.4533	2.1864	2.1281	1.9377	1.7717
Abies nephrolepis	An	0.5237	0.6916	3.0009	2.0257	3.1860	2.2442
Tilia spp.	T	0.2983	0.2938	1.4917	1.5820	3.5350	1.4281
Betula costata	Bc	1.0219	1.1296	2.1548	2.3771	2.0132	2.2986
Betula dahurica	Bd	1.4591	0.9864	2.8569	7.6667	4.5567	11.7714
Juglans mandshurica	Jm	0.8538	2.2995	4.6809	2.5693	6.2414	2.5274
Phellodendron amurense	Pa	1.0534	1.6035	7.0397	5.9343	7.8765	14.8661
Fraxinus rhynchophylla	Fr	0.8776	1.000	1.4701	3.1407	3.0405	3.1429
Populus davidiana	Pd	1.3169	1.2939	3.8120	1.8553	5.3103	3.4112
Ulmus pumila	Up	3.0375	0.7301	3.2849	4.5130	3.1731	9.0832
Quercus mongolica	Qm	1.5130	1.3048	0.4923	0.2815	0.3415	0.1920
Pinus koraiensis	Pk	0.7130	0.3609	0.9070	4.2567	3.7867	4.1054
Acer mono	Am	0.2443	-1.9976	2.1216	2.7808	3.8798	3.4514
Fraxinus mandshurica	Fm	2.4114	2.5841	1.1744	0.6941	1.3006	1.9525
Picea spp.	P	0.6649	0.8182	0.3827	2.2018	2.2394	1.8928
Larix olgensis	Lo	2.0385	1.0246	0.1756	0.2256	0.1848	0.2052

The interaction coefficients by the resource exploitation competition of *B. dahurica*, *Tilia* spp., *F. rhynchophylla*, *U. pumila*, *P. koraiensis*, *A. mono* and *Picea* spp. increased from 1986 to 1994 (*Table 1*), but it decreased for *A. nephrolepis*, *J. mandshurica*, *P. amurense*, *P. davidiana*, *Q. mongolica* and *F. mandshurica*. Only the interaction coefficients of *B. platyphylla* and *L. olgensis* changed slightly.

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The mean crowding coefficient increased for B. costata, B. dahurica, P. amurense, U. pumila, P. koraiensis and F. mandshurica (Table 1), and it decreased for A. nephrolepis, Tilia spp., J. mandshurica, P. davidiana, Q. mongolica, A. mono and Picea spp. But for B. platyphylla, F. rhynchophylla and L. olgensis it changed only slightly.

Spatial change of interspecific interaction for B. dahurica and P. koraiensis

For the sake of simplicity, here only the spatial change of multispecies interactions for *B. dahurica* and *P. koraiensis* at each 50 km was shown, respectively (*Fig. 1*). *B. dahurica* was chosen because its overall interaction with other species over the entire study area did not change significantly, but its spatial distribution of multispecies interactions changed from 1986 to 1994. *P. koraiensis* is an important species for industrial timber and a keystone species in the vegetation of this area. The scaling exponent of *B. dahurica* was 0 at 0-200 km in 1994, there was a higher change of interspecific interaction at this area. The interaction strengths of the same species at different locations were different. For *P. koraiensis* the interaction strength changed at 0-50 km and 250-300 km. The sudden change of multispecies interactions may provide information of disturbances and dramatic environmental change.

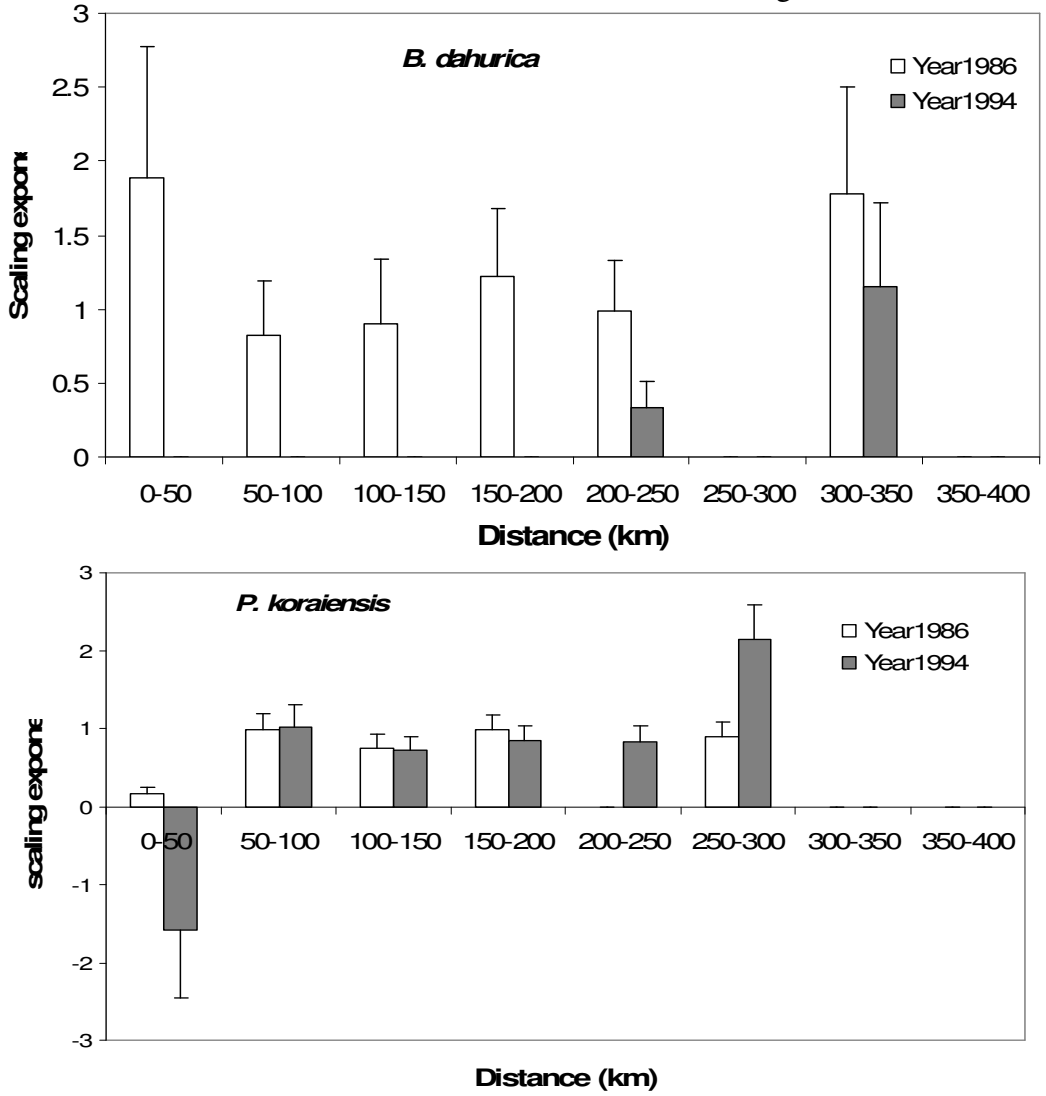

Figure 1. *The spatial distribution of multispecies interactions of B. dahurica and P. koraiensis at each 50 km along NECT by Taylor's power law.*

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The spatial distribution of species interaction coefficients estimated by resource exploitation competition changed for both *B. dahurica* and *P. koraiensis* (*Fig. 2*). For *B. dahurica* the species interaction coefficients increased at 200-250 km and 300-350 km. This is consistent with the result from scaling exponent. For *P. koraiensis* the interaction coefficients changed little at 0-50 km, but it increased beyond 100 km.

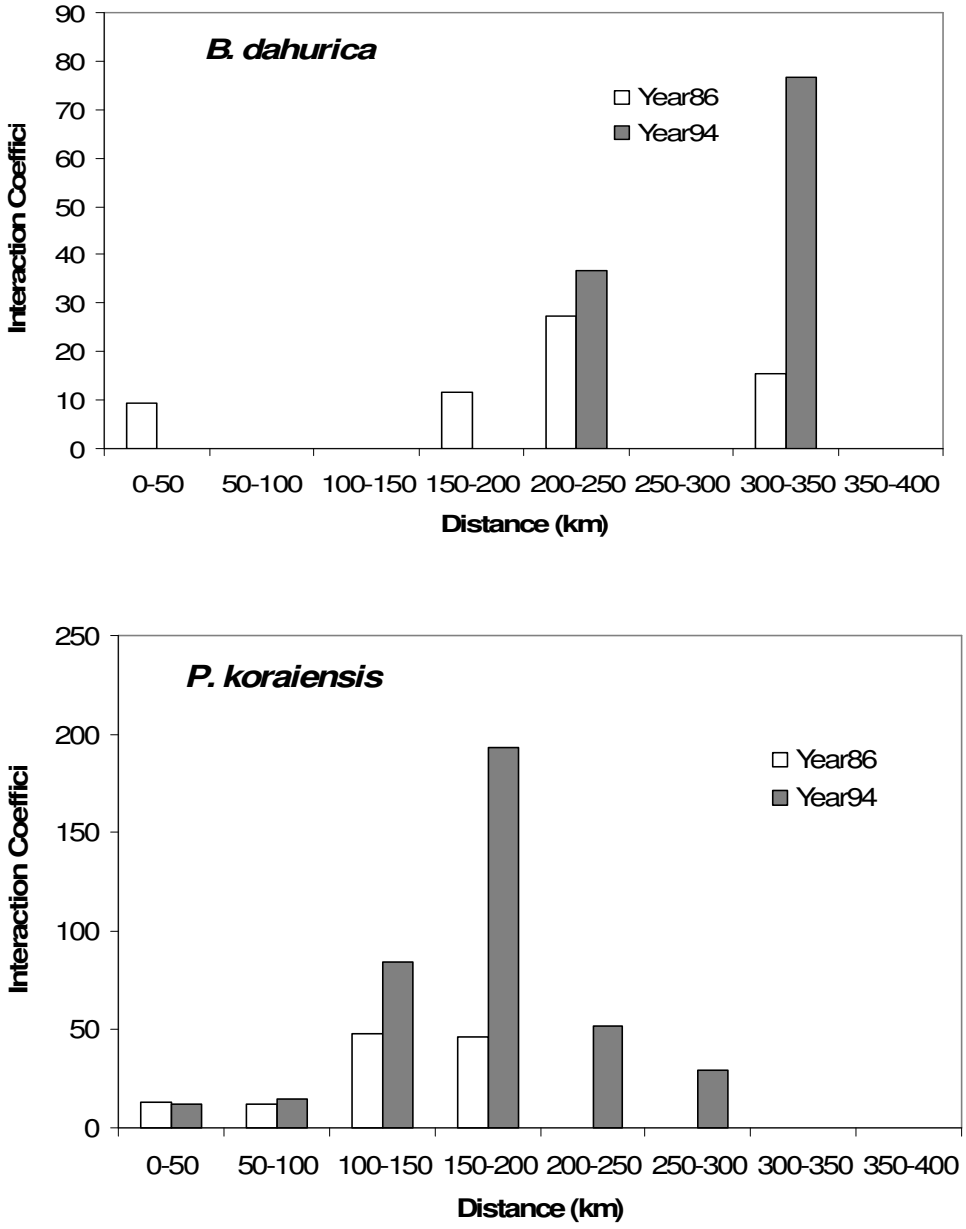

Figure 2. *The spatial distribution of multispecies interactions of B. dahurica and P. koraiensis at each 50 km along NECT by species competition coefficient.*

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For the spatial distribution of mean crowding coefficient of *B. dahurica*, the mean crowding coefficient decreased at 0-200 km and increased slightly at 300-350 km (*Fig. 3*). For *P. koraiensis* it decreased at 50-100 km and increased at 0-50 and 150-300 km. Most of these results are consistent with the other two methods.

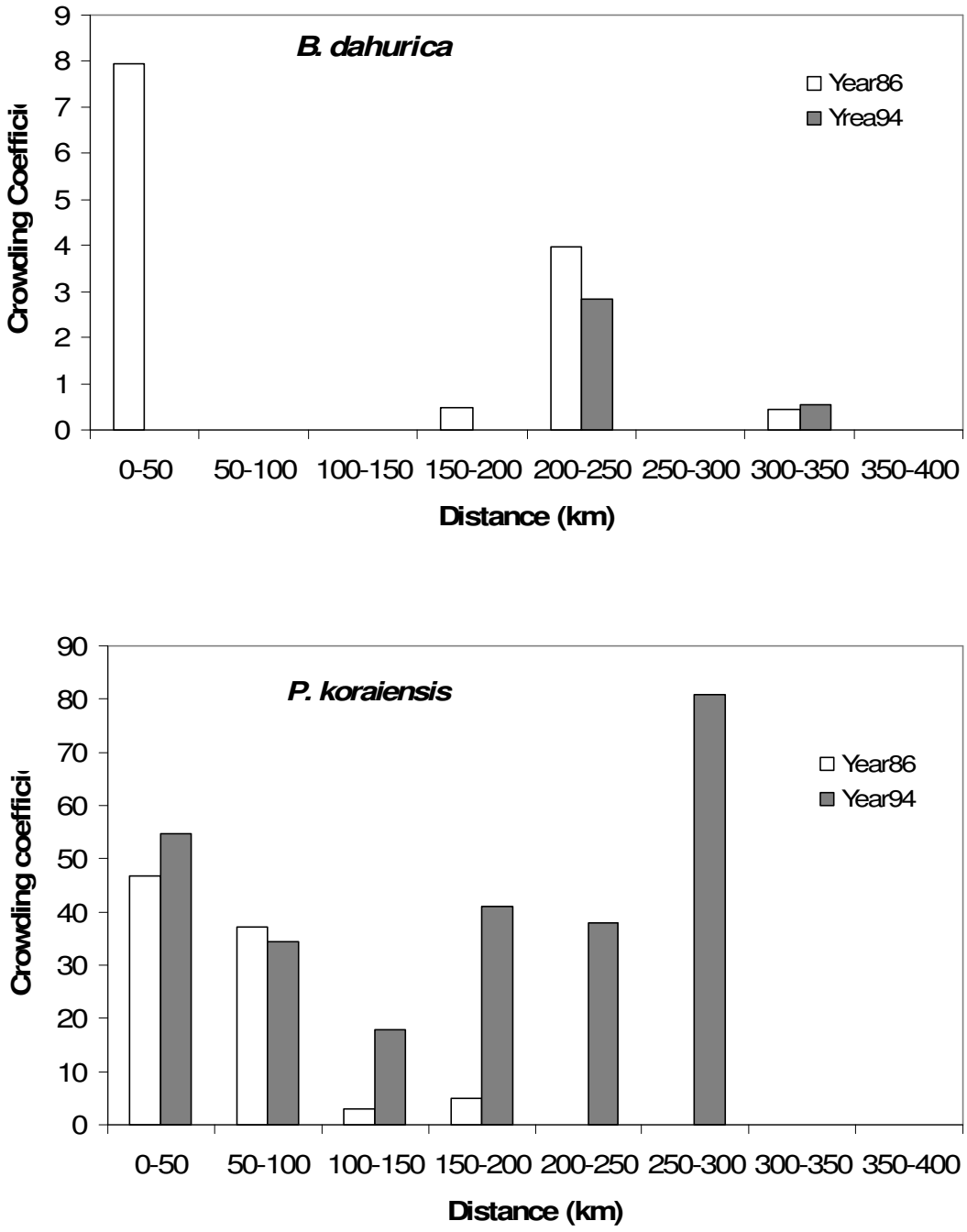

Figure 3. *The spatial distribution of multispecies interaction of B. dahurica and P. koraiensis at each 50 km along NECT by mean crowding measurement.*

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Discussion

There are differences and similarities between the estimates of species interaction using these three methods. The scaling exponent of Taylor's power law demonstrated that the multispecies interaction for *J. mandshurica*, *U. pumila*, *P. koraiensis*, *A. mono* and *L. olgensis* changed, while for most other species the interaction did not change significantly. As competitive interactions increase between species, the average scaling exponent decreases from 2 to 1 [17]. The possible explanations for the insensitivity of most species are the most abundant species often has a higher carrying capacity and/or is a superior competitor, it experiences weaker interspecific competition and has lower variability; but the variability in the rare species from other species' competition is relatively large, and this substantially increases the variance in the abundance of the rare species [17]. In this study, the relative percentages of abundance for each species in 1986 and 1994 are shown in *Table 2*. *J. mandshurica*, *P. amurense*, *F. rhynchophylla*, *P. koraiensis* and *Picea* spp. had a lower relative percentage of abundance, respectively; While *L. olgensis*, *U. pumila* and *A. mono* had a higher relative percentage of abundance, respectively. *B. dahurica*, *F. rhynchophylla*, *Q. mongolica*, *Tillia* spp. and *A. nephrolepis* had a higher change in the relative percentage of abundance in comparison with the others. However, by Taylor's power law it can be inferred that *J. mandshurica*, *P. koraiensis*, *L. olgensis*, *U. pumila* and *A. mono* could be described as species that experienced higher interaction because their scaling exponents changed significantly. The interaction strength was also changed for *A. nephrolepis*, *B. costata*, *P. amurense*, *F. rhynchophylla*, *F. mandshurica*, *Picea* spp., *B. platyphylla*, *B. dahurica*, but the change was not significant. The species with a higher change in the multispecies interaction estimated by Taylor's power law also had a change in the relative percentage of abundance (*Table 2*). But a species with a change in the relative abundance may not change its interaction with all other species because other species may also change their abundances during the same time period. Therefore, it is not enough to simply measure the abundance of each species over time in our current monitoring. Our results indicated that not all rare species experienced higher interaction from other species and environment. *A. mono* and *Tilia* spp., which were not rare species, also experienced a higher interaction from all others in 1986 and 1994. This result may be useful to identify the rare species that experienced high multispecies interaction or the species affecting the rare species. It has great implications for rare species preservation and management.

Using the species resource exploitation competition coefficients, it was found that all species had changed in multispecies interactions between 1986 and 1994 except *B. platyphylla* and *L. olgensis*. The interaction strength changed more for *B. dahurica*, *P. koraiensis*, *J. mandshurica*, *P. davidiana*, *Picea* spp. and *F. rhynchophylla*. Because this method is based on the species relative percentage of abundance, it assumes that species with a lower relative percentage of abundance experienced a relative higher multispecies interaction pressure. Some species which had changed the strength of multispecies interactions also changed their relative abundance, such as *A. nephrolepis*, *J. mandshurica*, but some did not. By using the interspecies mean crowding measurements, it was found that *B. dahurica*, *J. mandshurica*, *P. amurense*, *Tilia* spp., *P. davidiana* and *U. pumila* had higher degree of change in multispecies interaction coefficients. This result is similar with the result estimated by the species resource exploitation competition coefficients. Some species which had changed their

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multispecies interaction changed their relative abundance, such as *A. nephrolepis*, *J. mandshurica*, but some did not.

Table 2. *Relative abundance of each species along NECT in 1986 and 1994*

Tree species	Short names	Relative abundance (%)	
		1986	**1994**
Betula platyphylla	Bp	3.0466	4.2061
Abies nephrolepis	An	2.5538	4.2061
Tilia spp.	T	12.4104	14.1341
Betula costata	Bc	3.4498	4.4714
Betula dahurica	Bd	4.3907	0.6063
Juglans mandshurica	Jm	1.8817	2.2357
Phellodendron amurense	Pa	2.1057	1.2884
Fraxinus rhynchophylla	Fr	2.1505	0.5305
Populus davidiana	Pd	3.2258	2.3873
Ulmus pumila	Up	6.3620	5.8734
Quercus mongolica	Qm	37.6792	35.7711
Pinus koraiensis	Pk	2.6433	3.4104
Acer mono	Am	6.3620	7.5028
Fraxinus mandshurica	Fm	3.2706	2.8420
Picea spp.	P	2.7330	3.8272
Larix olgensis	Lo	5.7348	6.7071

Because of the climate gradients and species adaptation to these gradients, the distribution of species and their functional group is also different along NECT [4, 6]. Species preferring moisture grow in the eastern part of the study area (moist end from beginning) (e.g., *P. koraiensis*); in contrast, species preferring dry conditions usually appear in the western part (dry end) (e.g., *Q. mongolica*). Here only two species were explained in details for their spatial distribution of interactions. The spatial distribution of multispecies interaction of *B. dahurica* and *P. koraiensis* changed in different areas from 1986 to 1994. With no interspecific competition in resources exploitation for *B. dahurica* at 0-200 km, its scaling exponent and crowding coefficient both became 0. *B. dahurica* may die out more easily because of (i) its shade-intolerance at early succession stages [3]; (ii) competition from other tree species [3]; (iii) drought and high air temperature [6]; and (iv) insects or pathogens [22]. At the distance of 200-250 km and 300-350 km the competition for resources increased, and the scaling exponent decreased; but its crowding coefficient decreased at 200-250 km and only increased slightly at 300-350 km. For *P. koraiensis* the competition coefficient in resource exploitation increased at 50-300 km, and its crowding coefficient also increased, but its scaling exponent became negative at 0-50 km and more than 2.0 at 250-300 km, respectively. By analyzing the spatial change of scaling exponent, interspecific competition coefficient and crowding coefficient at different scales, we can monitor the interspecific interaction of tree species. However, the biological meaning of scaling exponent that is less than 0 and more than 2.0 is still not straightforward. Disturbances, such as wind damage and pathogens, frequently occur in this area just like any forests,

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but the main factor is precipitation change [6]. At some locations land use was changed during this time period, but it did not occur in the area of these permanent plots.

Serious drought can cause some trees to die or to be easily damaged by disturbances (e.g., wind). Disturbances can change the species interaction by changing interspecies competitions and environmental conditions. These measurements reflected changes in species viability and also year-to-year variability. Sometimes year-to-year variability is very important for species viability. The way to analyze this kind of variability is to use the combination of three methods and use long term data. Therefore, the combination of three measurements can reflect the change in species viability at different areas along NECT.

The different results from each of the three methods may be primarily due to the fact that the scaling exponent of Taylor's power law considered interactions both from species and environment; whereas, the species resource exploitation competition coefficients and the interspecies mean crowding did not consider both. The species resource exploitation competition coefficient considers only competition for resources and is a measure of exploitation competition under the assumption of no inference [10]. The interspecies mean crowding indicates the extent of crowding [14]. The relative percentage of abundance is mainly considered in the species resource exploitation competition coefficient, and real abundance is considered by mean crowding methods. *L. olgensis* received more competition from other species using the scaling exponent of Taylor's power law, but it was considered little competition and no significant change by the species resource exploitation competition coefficients and mean crowding methods. The species resource exploitation competition coefficients and mean crowding methods both determined that *P. davidina* and *Q. mongolica* decreased interactions with others, but the method of scaling exponent of Taylor's power law did not (*Table 3*).

Table 3. *Comparison of the results from the three methods (* indicates that this species is also detected by at least another method)*

Method	Decreased interaction strength	Increased interaction strength
Taylor's power law	*J. mandshurica**, *A. nephrolepis**, *B. costata*, *P. amurense**, *F. rhynchophylla*, *F. mandshurica**, *Picea* spp.*	*U. pumila**, *P. koraiensis**, *A. mono**, *L. olgensis*, *B. platyphylla*, *B. dahurica**
Species competition coefficient	*A. nephrolepis**, *J. mandshurica**, *P. amurense**, *P. davidiana**, *Q. mongolica**, *F. mandshurica**	*B. dahurica**, *Tilia* spp., *F. rhynchophylla*, *U. pumila**, *P. koraiensis**, *A. mono**, *Picea* spp.
Mean crowding	*A. nephrolepis**, *Tilia* spp., *J. mandshurica**, *P. davidiana**, *Q. mongolica**, *A. mono*, *Picea* spp.*	*B. costata*, *B. dahurica**, *P. amurense*, *U. pumila**, *P. koraiensis**, *F. mandshurica*

Therefore, the three methods describe the different perspectives of species interactions, but also have relationships with each other. The increased interactions between multiple species may result in change in relative percentage of abundance or may not, but with a relative lower percentage of abundance, such as with rare species,

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extinction may occur in this area. By combining the results of three methods, it may be helpful to monitor species viability under the interactions with other species and environment and provide management strategies for species monitoring and conservation. The species with increased interactions by the three methods need higher priority to monitor and manage, such as *U. pumila*, *P. koraiensis* and *A. mono*. If species with increased interactions were determined by the scaling exponent of Taylor's power law, but were not detected by the other methods, then, the species may experience more pressure from environment. Species without increased interactions determined by one of the methods, but were detected to have increased interactions by the other two methods, may be at medium priority to manage, such as *L. olgensis* and *B. dahurica*. Species without detected interactions by any of the three methods may be at low priorities to manage. The combination of these three methods may be useful for monitoring the spatial and temporal dynamics of species under multispecies interaction if combined with environmental information, and provide some practices for conservation (e.g., control the abundance of certain species with higher interspecific interaction). But the biological meaning of a scaling exponent less than 0 and more than 2.0 is still not clear, and its relationships with resource competition and mean crowding require further study. Due to the data limitation we can not test our results here. More research remains to be done to test whether this method can be applied to other ecosystems or systems with more than one trophic level, such as animal-plant interaction.

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ADSORPTION OF PHENOL FROM AQUEOUS MEDIA BY AN AGRO-WASTE *(HEMIDESMUS INDICUS)* BASED ACTIVATED CARBON

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Abstract. The adsorption of phenol by an agro-waste based activated carbon prepared from the root residue of Hemidesmus Indicus (HIC) was investigated to assess its possible use as adsorbent. The effect of various factors, namely, pH, initial adsorbate concentration, adsorbent dosage and contact time were studied to identify adsorption capacity of HIC. The results were compared to that obtained from adsorption of phenol by commercial activated carbon (CAC). Adsorption data were modeled with the Langmuir and Freundlich isotherms. The kinetic models were also applied for the pseudo-first-order, pseudo-second-order, intra-particle diffusion and pore diffusion coefficients. Although HIC and CAC showed much similar isotherm models and kinetics, yet HIC was found to show much higher boundary layer effect and pore-diffusion coefficients, in relation to CAC.
Keywords: *Phenol, agro-waste, adsorption, Isotherms, adsorption kinetics*

Introduction

Since 1860, phenol has been in production, with its basic use as an antiseptic. During late 19th century and thereafter the use of phenol has been further extended to the synthesis of dyes, aspirin, plastics, pharmaceuticals, petrochemical and pesticide chemical industries. In fact, by 2001, the global phenol production has reached an impressive 7.8 million tons [1].

Normally, discharges from the aforesaid phenol-yielding industries find their way to water bodies and subsequently affect the water quality adversely. As per the studies by various authors, phenols and its derivatives have been reported to cause undesirable and deleterious effect in water, even at a concentration as low as 0.1 ppm [2]. In fact, the adverse effects of phenol have been observed on the central nervous system, cardiovascular system as well as urino-genital systems of human being, often expressed by the multiple symptoms: convulsions, coma, cardiac disorders, respiratory failure and collapse [3]. As per literature, the various analytic methods attempted for dephenolation of wastewater include steam stripping, solvent extraction, oxidation (O_3, H_2O_2, and ClO_2), ion exchange, biodegradation and adsorption methods [4, 5, 6]. Out of all these, adsorption methods are the most-widely used techniques and activated carbon has been the predominant adsorbent all over the world [7, 8]. However, due to its high cost and low regeneration capacity, since the last three decades, extensive researches have been directed towards investigating the adsorption characteristics and potentials of cheaper materials and solid wastes, such as fly ash, peat, soil, rice husk, sawdust, bagasse and so forth [9, 10, 11, 12].

In the present study, the extracted residue of a root of an Indian plant, namely *Hemidesmus indicus* (also called Sveta sariva, Ananda mul or Nannari and is popularly used as energy stimulant soft drink), is studied for its adsorption capacity. In fact,

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Hemidesmus indicus has been known to possess various favourable medical characteristics, such as promoting good circulation, clearing toxins, balancing the glandular system, regulating hormone secretion, providing calories, protecting against UV-radiation, stimulating metabolism and curing several stomach and kidney disorders. It has also been indigenously used for water purification (mainly turbidity removal) in certain traditional communities in India. However, the residue of the root of the plant has been considered wastes, with apparently no specific utilization (probably, except combustion and composting to a limited degree). The present paper is aimed at evaluation of the effectiveness of *Hemidesmus indicus* root residue and determination of its optimization condition for phenol-sorption.

Experimental Methods

Preparation of activated carbon

Roots of *Hemidesmus indicus,* a locally available slender twining herb, was collected and after extraction of its juice by traditional method (of grinding, boiling with water and filtering), was cleaned and washed thoroughly to remove water-soluble substances (so as not to affect the aqueous characteristics of phenolated wastewater). The material was acidulated using 1:1 wt ratio of concentrated H_2SO_4 and subsequently allowed to soak for 24 hours at room temperature. Thereafter, the sample materials were placed in an oven and heated to 200 $^{\circ}$C for 24 hours, followed by cooling, washing (with distilled water) and soaking with 1% $NaHCO_3$ solution (to remove any excess acid). The washing with distilled water was continued till the uniform characteristics of both filtering and filtered water (with respect to colour, pH and turbidity). The thoroughly washed sample was dried and subsequently subjected to pyrolosis at 850–900 $^{\circ}$C for 30 minutes. The samples, thus obtained, were ground in a ball mill and the particles having an average diameter of 0.5 mm (using the sieves of 20–50 mesh, ASTM), were collected and stored for further studies.

Characterization of Activated Samples

Activated carbon, obtained as above, was characterized by adopting the standard procedures [13, 14, 15]. The moisture content of the carbon was determined by heating a known weight of the sample in an air oven maintained at 105 ±5 °C for about 4 h. The residue was ignited in a muffle furnace at 1000^0C for about 3 h to determine the ash content. Surface acidity of the sample was studied using boiled water and decolourising power, using methylene blue solution. The adsorption characteristics was studied in terms of phenol number (the amount of carbon required for 90% removal of phenol, which indicates the ability to remove taste and odour of the activated carbon) and iodine number (the ability of the carbon to adsorb low molecular weight substances, defined as the milligrams of iodine (I_2) that are adsorbed per gram of carbon when the equilibrium concentration of the bulk saturation (C_{eq}) is 0.02 N). The surface area of the activated carbons was carried by BET (Brunauer Emmett Teller) Nitrogen adsorption method. The characteristics of the activated carbons are presented in Table 1.

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Table 1. *Characteristics of HIC and CAC*

Property	**HIC**	**CAC**
Bulk Density ($g.ml^{-1}$)	0.48	0.65
Moisture content (%)	10.35	12.57
Ash content (%)	2.08	2.78
Fixed Carbon (%)	97.92	97.22
Solubility in water (%)	0.80	1.55
Solubility in 0.25M HCl acid (%)	1.2	3.0
pH of 5% slurry	6.3	8.2
Decolourising power ($mg.g^{-1}$)	82	74
Phenol number (mg)	5.3	5.2
Iodine number ($mg.g^{-1}$)	204	192
Surface area ($m^2.g^{-1}$)	627	596

Batch experiments

A 100 ml each of synthetic phenol solution (30 ppm) adjusted to different pH values by 0.1 N NaOH or 0.1 N H_2SO_4, were placed in 250 ml leak-proof reaction bottles and a known amount of *Hemidesmus indicus* carbon (HIC) was added to each bottle. The solution was equilibrated for 24 hours at room temperature, followed by filtration of the adsorbent and subsequent analysis of filtrate for phenol concentrations were determined by spectrophotometric analysis of the colour resulting from the reaction of phenol with 4- aminoantipyrine at a wavelength of 500 nm [13]. Kinetic experiments were conducted using a known weight of carbon dosage for a phenol concentration in the range of 5–40 ppm. Periodically, after regular intervals of time, samples were analysed for phenol concentration. The rate constants were calculated by using the various models (viz. pseudo-first-order, pseudo-second-order, intra-particle diffusion and pore diffusion coefficients).

Results and Discussion

The adsorbent prepared from HIC was studied for adsorption and the results thus obtained were compared with that of CAC and the removal efficiency of phenol from aqueous solution was estimated, under different experimental conditions.

Effect of carbon dosage

Figure 1 shows the amount of phenol removed as a function of carbon dosage from the solution at neutral pH. Carbon dosage was varied from 5 to 500 mg and equilibrated for 24 hours. As evident from the figure, to remove the entire amount of phenol (with initial concentration of 30 ppm), the minimum adsorbent dosage required was found to be 35mg of CAC or 30mg of HIC. Thus, HIC seems to be better dephenolating agent than CAC.

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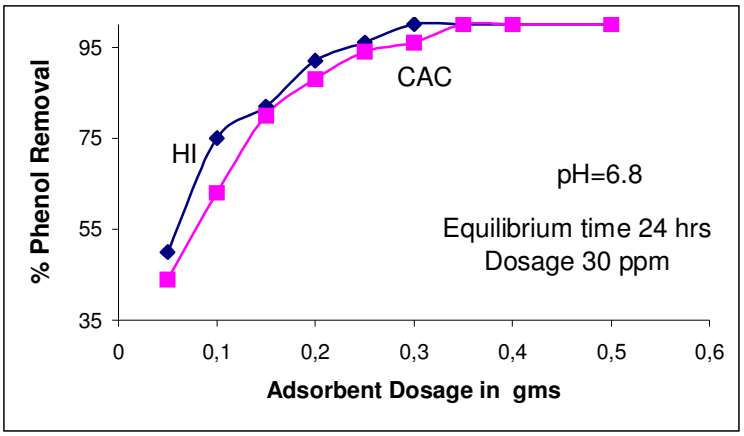

Figure 1. *Effect of Adsorbent Dosage*

Effect of pH on phenol adsorption

Since phenolsorption has been reported to be affected by the pH of the adsorbate [9], the adsorption of phenol by HIC and CAC were studied at various pH values of the phenol solution (100ml, 30-ppm). The amount of phenol adsorbed shows a declining trend with higher as well as with lower pH, with maximum removal of phenol (up to almost 100 % by both the adsorbents) at neutral pH (Figure 2). This reduction of phenol-sorption may be because of the suppression by hydrogen ions (at lower pH), and hydroxyl ions (at higher pH) in addition to formation of various phenolic compounds at both acidic and alkaline conditions [16, 17].

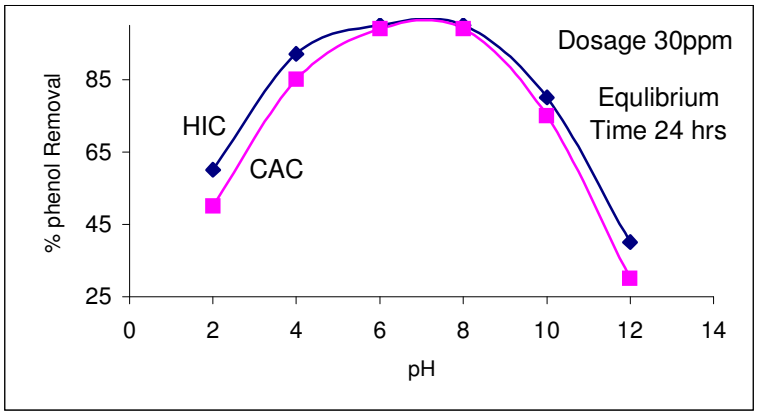

Figure 2. *Effect of pH on Phenol Adsorption*

Effect of contact time

Figure 3 shows the effect of contact time on the removal of phenol by HIC and CAC. For different duration of time (ranging from 0.5 to 24 hours), well-shaken phenol solution (100 ml, 30 ppm) was studied for equilibrium. The study revealed 5 hour and 6 hours as the equilibrium time for HIC and CAC, respectively.

Adsorption Isotherms

In order to determine the adsorption potential of both the adsorbents (viz. HIC and CAC) for the removal of phenol, study of adsorption isotherm was carried out and

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tested against the Freundlich and Langmuir isotherm models, using standard procedures used by various authors [9, 18, 19].

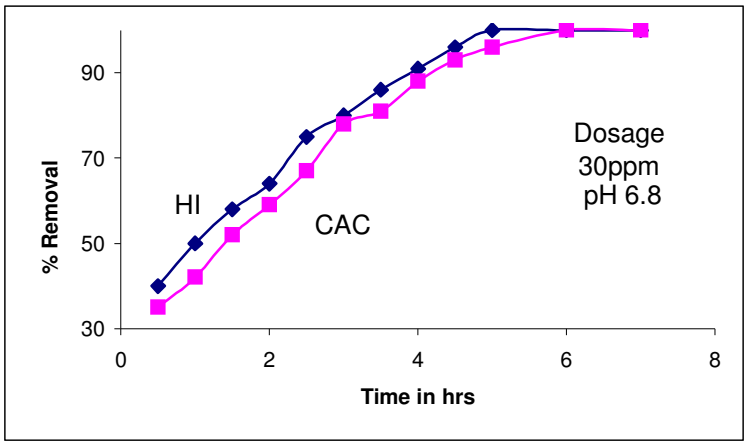

Figure 3. *Effect of Time on phenol Adsorption*

Langmuir Isotherm:

The Langmuir equation is given as:

$$\frac{C_e}{q} = \left(\frac{1}{Q_0 b}\right) + \left(\frac{C_e}{Q_0}\right) \qquad \text{(Eq.1)}$$

Where, q = x/m, represents the amount of adsorbate adsorbed per unit mass of adsorbent (mg/g) and 'C_e', 'Q_0 and 'b' refer to the equilibrium concentration (ppm), monolayer adsorption capacity (mg/g) and surface energy (g/l), respectively. A plot of Ce/q verses Ce as shown in Figure 4, where '$1/(b\ Q_0)$' and '$1/Q_0$' correspondingly represent the intercept and slope (comparing with the normal equation for straight line as 'y = mx + c').

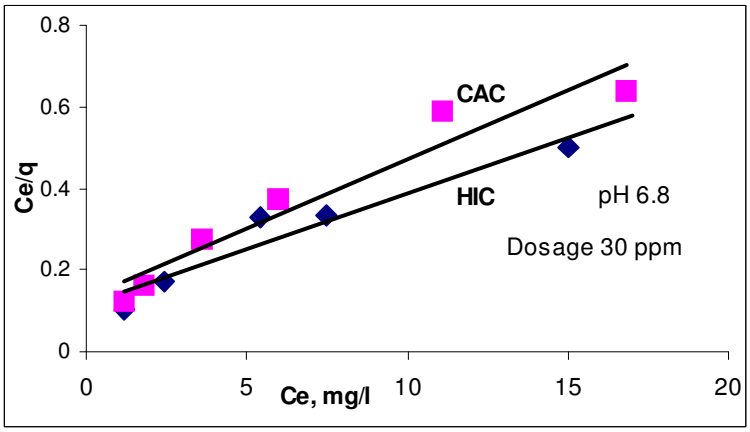

Figure 4. *Langmuir Adsorption Isotherm for Phenol*

The Langmuir isotherm can also be expressed by a separation factor (R_L), which is given by the equation [20].

$$R_L = 1/\ (1 + b.\ C_i) \qquad \text{(Eq.2)}$$

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Where, 'C_i' is the initial concentration of phenol in ppm and '**b**' is the Langmuir constant in g/l. The separation factor 'R_L' indicates the nature of the adsorption process as given in Table 2.

Table 2. *The process nature of separation factor.*

S.No.	R_L Value	Type of process
1	$R_L > 1$	Unfavourable
2	$R_L = 1$	Linear
3	$0 < R_L < 1$	Favourable
4	$R_L = 0$	Irreversible

Freundlich Isotherm: The Freundlich equation is expressed as

$$\frac{x}{m} = q_e = kC_e^{1/n} \qquad \text{(Eq.3)}$$

Where, '**k**' and '**n**' are the measures of adsorption capacity and intensity of adsorption. 'q_e' is the amount of phenol adsorbed per unit mass of adsorbent and 'C_e' is the equilibrium concentration in ppm. The logarithmic form of **Freundlich** equation can be expressed by

$$\log(x/m) = \log q_e = \log k + 1/n \log C_e \qquad \text{(Eq.4)}$$

From the straight line (obtained by plotting '**log (x/m)**' against '**log C_e**' as shown in Figure 5 the corresponding slope and intercept can be determined from '**1/n**' and '**log k**' values. Table 2 indicates the values of Langmuir and Freundlich constants.

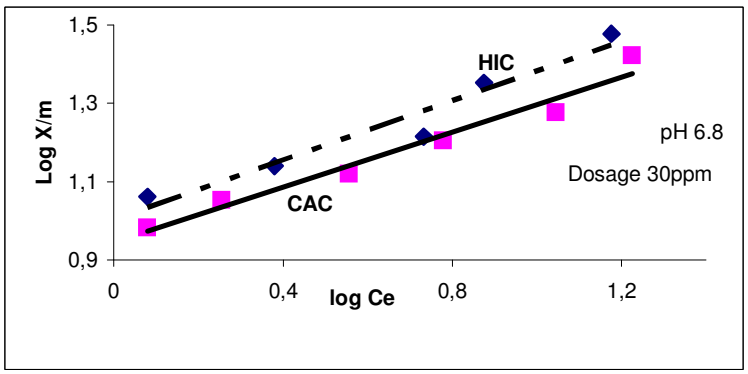

Figure 5. *The Freundlich Adsorption Isotherm for Phenol*

The results reveal that the adsorption of phenol on HIC and CAC obeys both Freundlich and Langmuir adsorption isotherms, as indicated by high R^2 Values (>92%). Greater the value of **k** (the Freunlich constant), higher the phenol uptake from aqueous solution [9,18]. As shown in Figure 5, higher ***k***-value of HIC (1.04 mg/g) indicates greater affinity for phenol compared to that of CAC (0.57 mg/g), which shows better effectiveness of the phenol-HIC system than phenol-CAC system. The adsorption

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intensity, '**n**' is found to be 2.65 and 2.84 for HIC and CAC, respectively. It is observed that, in both these systems, the **n**-values satisfy the condition(s) of heterogeneity, i.e., 1< n < 10 as well as 0 < 1/n < 1 [16]. The higher magnitude of 'Q_0' indicates that the amount of phenol per unit weight of sorbent (to form a complete monolayer on the surface) seems to be significantly higher for phenol–HIC and phenol–CAC systems. A relatively lower '**b**' – value (< 0.3) implies low surface energy in both the systems, thus indicating a probable stronger bonding between phenol and sorbents [18]. In fact, fairly low to moderate '**b**' - values have been reported in many of the sorbent-phenol systems, involving palm-seed-coat-activated-carbon, bentonite, and rice husk [9, 11, and 19].

Adsorption kinetics

Kinetics of adsorption, a standard analysis in defining adsorption efficiency, describes the solute uptake rate, which in turn governs the residence time of adsorption reaction. The study the adsorption kinetics of the two phenol-sorbent systems (viz. -HIC and -CAC) were carried out using batch experiments. The adsorbents (0.3g) were separately exposed to the synthetic phenol solution (100ml, 30ppm) and the amount of phenol adsorbed was estimated for a time period of 30 min to 360 min. (or, 6 hours, which refers to the equilibrium time, Figure 1.)

Table 3. *Details of Isotherm Constants*

Name of Adsorbent	**Langmuir Constants**			**Freundlich Constants**			**Separation Factor**
	Q_0	b	R^2	k	n	R^2	R_L
Hemidesmus Indicus Carbon (HIC)	370.37	0.23	0.92	1.04	2.65	0.94	0.12
Commercial Activated Carbon (CAC)	294.11	0.26	0.93	0.57	2.84	0.96	0.11

Pseudo- First – Order Kinetics: A widely-used Lagergren model was employed to study the pseudo first order kinetics [21,22, 23].

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \qquad \text{(Eq.5)}$$

where '$\mathbf{q_e}$' and '$\mathbf{q_t}$' refers to the amounts of phenol (ppm) adsorbed on the activated carbon at equilibrium time and time **t** (min) and $\mathbf{k_1}$ as rate constant (min^{-1}), respectively .

Integrating the above equation between the limits from t = 0 to t = t and from $q_t = 0$ to $q_t = q_t$

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t \qquad \text{(Eq.6)}$$

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Thus, the rate constants (**k_1**) were obtained from slope of the plots of **log (qe-qt)** Vs **t** (R^2 > 0.9, Eq.6) and presented in Table 4. HIC was found to be better rate-limited (0.0104) by pseudo-first order model, than CAC (0.0092).

Table 4. *Rate constants for the phenol removal by HIC and CAC*

Type of Carbon	Pseudo-First-Order model			Pseudo-Second-Order model		
	qe	R^2	$k_1(min^{-1})$	k_2(g/mg.min)	h(mg/g.min)	R^2
Hemidesmus Indicus Carbon(HIC)	16.10	0.93	0.0104	0.0003	0.4166	0.97
Commercial Activated Carbon(CAC)	11.01	0.94	0.0092	0.0002	0.3246	0.94

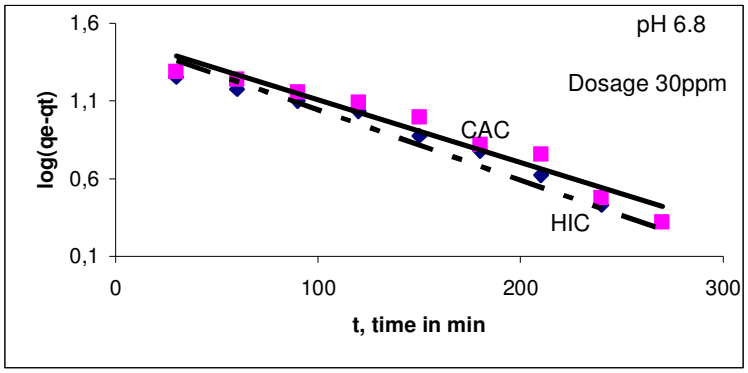

Figure 6. *Pseudo-First-Order Kinetics for HIC and CAC*

Second order Kinetics:

The sorption data were also studied by second order kinetics [24,21]

$$\frac{dq_t}{dt} = k_2 (q_e - q_t)^2 \qquad \text{(Eq.7)}$$

Where, '**k_2**' refers to the rate constant of second order adsorption (g/mg.min).
On Integration,

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad \text{(Eq.8)}$$

Expressing in the form of a standard straight-line equation ($y = mx + c$);

$$\frac{t}{q_t} = \frac{1}{h} + \frac{t}{q_e}, \text{ '}h\text{' being } k_2 q_e^2 .$$

The plot of **t/qt** vs **t** (Figure 7) gives a linear relationship (R^2 > 0.9), from which the constants **k_2, q_e** and **h** were determined (Table 3). As in the case of the pseudo-first order model, the present analysis also indicate HIC as marginally better rate-limited (0.0003) by pseudo-second order model, than CAC (0.0002).

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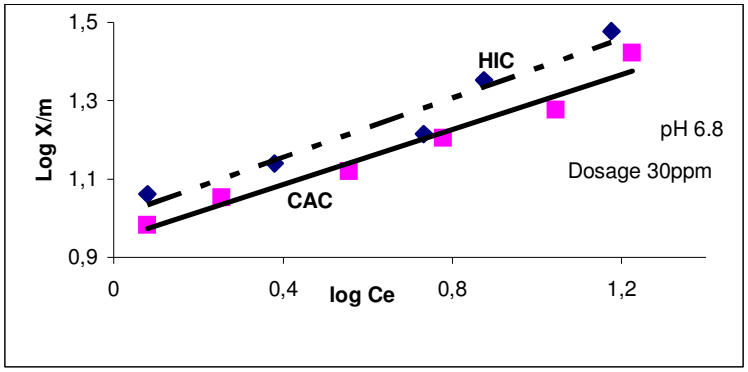

Figure 7. *Pseudo-Second-Order Kinetics for HIC and CAC*

Intra-particle diffusion

The structure of the adsorbent and its interaction with the diffusing adsorbate (Intra-particle diffusion) influence the rate of transport, where the solute movement is a function of concentration gradient [25] and the rate constant (ki) can be determined by Intra-particle diffusion model [26].

$$q_t = k_i\, t^{(0.5)} + C \qquad \text{(Eq.9)}$$

Where, $\mathbf{q_t}$ refers to the amount of phenol adsorbed in mg/g at time, **t**; intercept **C**, indicating the boundary layer effect [27] and $\mathbf{k_i}$, the Intra-particle diffusion rate constant (mg/g. $min^{1/2}$). A plot between the amount of phenol adsorbed and square root of time gives the rate constant (Figure 8, Table 4). The results indicate the intra-particle diffusion as a rate-determining step for HIC and CAC. The boundary layer effect of HIC (5.19) was found to be more than twice to that of CAC (2.16).

The pore diffusion coefficient, D, for the removal of phenol by HIC and CAC were calculated (assuming a spherical-geometry of the adsorbents; 20-50 ASTM; average mesh size 0.5mm):

$$t_{1/2} = 0.03\, r_o^2 / D \qquad \text{(Eq.10)}$$

Where, $\mathbf{t_{1/2}}$ refers to the time for half adsorption (sec)**;** $\mathbf{r_0}$, the diameter of the particle (cm); **D**, the pore diffusion constant (cm^2/s).

Pore diffusion in the adsorbents (Table 5) is assessed to be less significant due to relatively higher values of the coefficients, in contrast to the rate-limiting range (10^{-11} to 10^{-13} cm^2/s) [28]. However, the pore diffusion coefficient for HIC (2.08×10^{-8} cm^2/s) shows almost 1.5 times that for CAC (1.39×10^{-8} cm^2/s).

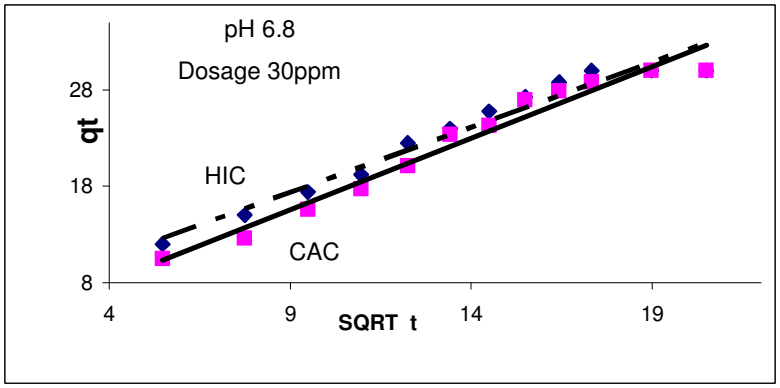

Figure 8. *Intra-particle Diffusion plot of phenol on HIC and CAC*

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Table 5. *Intra particle and pore diffusion constants*

Type of Carbon	**Intra-particle Diffusion model**			**Pore Diffusion Coefficient**
	C	R^2	k_i(mg/g. $min^{1/2}$)	D (cm^2/s)
Hemidesmus Indicus Carbon (HIC)	5.19	0.95	1.35	2.08×10^{-8}
Commercial Activated Carbon (CAC)	2.16	0.96	1.48	1.39×10^{-8}

Conclusions:

The present studies indicate *Hemidesmus indicus* Carbon (HIC) as a better adsorbent than the commercial activated carbon (CAC), as indicated by its higher adsorption at lower adsorption dosage (and period of equilibrium). Adsorption characteristics of HIC and that of CAC showed notable similarity as reflected by (i) their obedience to both Langmuir and Frueundlich isotherm models, (ii) similar rate-characteristics (in both first- and second- order kinetics, with correlation coefficients greater than 0.9), (iii) rate-determining character of intra-particular diffusion, and (iv) less significant role by pore-diffusion process. However, HIC was found to be marginally better rate-limited (than CAC) by both pseudo-first and pseudo-second order models. Besides, 'the boundary layer effect of' and 'the pore diffusion coefficient for' HIC was found to be almost double to and 1.5 times of CAC, respectively.

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SIMULATED ACID AZO DYE WASTEWATER TREATMENT USING SUSPENDED GROWTH CONFIGURED SEQUENCING BATCH REACTOR (SBR) UNDER ANOXIC-AEROBIC-ANOXIC MICROENVIRONMENT

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Abstract. Studies on the treatment of simulated acid metal complex azo dye (C.I.Acid black 210) wastewater was carried out in anoxic-aerobic-anoxic microenvironment using a periodically operated sequencing batch reactor (SBR) with suspended growth configuration at two organic loading rates (0.56 Kg COD/cum-day and 0.75 Kg COD/cum-day). The experimental data revealed the effective performance of the SBR system in the process of azo dye minerilazation, which may be attributed to the prevailing anoxic microenvironment present during the cycle operation. The reactor showed rapid startup (within 22 days) and stabilization (within 6 days after loading) compared to conventional continuously operated systems. Organic loading (shock loads) rates have shown relatively less effect on the performance.
Keywords: *Simulated acid azo dye wastewater, Sequencing Batch Reactor (SBR), periodic discontinuous process, suspended growth configuration, oxygen consumption rate.*

Introduction

Dyes are synthetic aromatic organic compounds, which are normally used for coloration of various substances. During textile processing, inefficiencies in dyeing result in large amounts of the dyestuff (varying from 2% loss when using basic dyes to a 50% loss when certain reactive dyes used) being directly lost to the wastewater, which ultimately finds its way into the environment [2-4]. Among all the chemical classes of dyes, azo dyes are considered to be recalcitrant, non-biodegradable and persistent [1]. Dye containing wastewater treatment is considered to be one of the challenging areas in the environmental fraternity. Eventhough physico-chemical methods are effective in dye removal, the overall cost, regeneration problem and associated problems of secondary sludge generation limits their application [5,6]. Dye containing wastewater is normally not amenable for conventional biological wastewater treatment due to the recalcitrant and inhibitory nature of the wastewater [7].

Biological treatment of dye containing wastewater is particularly challenging owing to the inhibition and/or toxicity of these compounds when they serve as microbial substrates. More recently researchers have been focusing their attention on the alternative approaches like periodic discontinuous process, which promotes the mineralization of toxic compounds. This is developed on the basic scientific assumption that periodic exposure of the microorganisms to defined process conditions is effectively achieved in a batch system and the exposure time, frequency of exposure and amplitude of the respective concentration can be set independent of any inflow condition [9]. In SBR mode operation the reactor volume varies with time, where as it

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remains constant in the traditional continuous flow system. From the process engineering point of view, the SBR system is distinguished by the enforcement of controlled short term unsteady state conditions leading in the long run to a stable steady state with respect to the composition and metabolic properties of the microbial population growing in the reactor by way of controlling the distribution and physiological state of the microorganisms [9]. Interest has been growing worldwide both in scientific research and in practical application of SBR technology. SBR has been successfully applied for the treatment of domestic wastewater, medium and lower strength landfill leachates, simulated dye wastewater's and contaminated soils [9-23]. This communication presents experimental data pertaining to the investigate carried out to treated simulated azo dye wastewater using suspended growth configured sequencing batch reactor (SBR).

Materials and Methods

Simulated azo dye wastewater

Acid Black EBR H/C (C.I Acid black 210), an acid dye belonging to azo (metal complex) chemical group was used in this study (gifted by M/s Atul Chemical Ltd, India). This dye was normally used for dyeing cotton and woolen fabric and has a solubility of 30 g/l at 90°C. The simulated dye wastewater was prepared prior to the experiments by dissolving the requisite amount of dye (25 mg/l and 35 mg/l) and glucose (1 g/l) in tap water along with 10 ml of nutrient solution (glucose-1 g; $MgSO_4.7H_2O$-0.22 g, $FeSO_4.7H_2O$- 0.5 g, $NiSO_4$. H_2O-0.02 g, $NaHCO_3$-5 g, NH_4Cl-1.3 g, KH_2PO_4-0.05 g, $CaCl_2$-0.058 g, $FeCl_3.6H_2O$-0.017 g, $MnCl_2$-0.007 g, $ZnCl_2$-0.004 g, $CoCl_2.H_2O$- 0.004 g, $NaBO_2.10H_2O$- 0.001 g dissolved in 1 l distilled water [pH-7.0]).

Reactor Operation

SBR was fabricated in laboratory using perplex glass material with a designed volume (total) of 1.0 liters and working volume of 0.8 liters. The details of reactor configuration were presented elsewhere[21]. The outlet of the reactor used for wastewater withdrawal was provided at 0.025-m length from the bottom of the reactor, which prevents biomass loss from the reactor after the settle phase is completed. The reactor was operated in suspended growth configuration in sequential batch mode at a constant room temperature (26±2ºC). SBR was operated with a total cycle period of 24 hours consisting of 30 minutes of fill phase, 23 hours of react (aerobic) phase with recycling, 15 minutes of settle phase and 15 minutes of decant phase.

Table 1. *Details of sequence phase variation during cycle operation*

Phase	Cycle period	Air supply	Recirculation	Microenvironment Condition
Fill	30 minutes	Off	Off	Anoxic
React	23 hours	On	On	Aerobic
Settle	15 minutes	Off	Off	Anoxic
Decant	15 minutes	Off	Of	Anoxic

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Various sequence phases in the SBR operation was controlled by pre-programmed timers (ETTS, Germany). Feed, decant and recirculation operations were carried out using peristaltic pumps (Watson Marlow 101 U/R). Recirculation at a rate of 1.6 l/day was maintained throughout the reactor operation to achieve a homogeneous distribution of substrate as well as uniform distribution of suspended biomass. During the reaction phase, aqueous phase dissolved oxygen (DO) was maintained in the range of 2.5 to 3.0 mg/l. The pH of the influent feed was adjusted to 7.1±0.1 before feeding. The reactor was inoculated with aerobic biomass (VSS: 2.7 g/l) acquired from an operating laboratory scale SBR unit treating complex chemical wastewater. After inoculation, the reactor was operated with designed synthetic feed (organic loading rate (OLR) of 35 Kg COD/cum-day) to build up the biomass. When the reactor approached VSS concentration of 2.5 g/l, simulated dye wastewater at an OLR of 0.35 Kg COD/cum-day was loaded and subsequently after stable performance was achieved, the reactor was further operated at higher OLR (0.75 Kg COD/cum-day).

Analytical Protocols

Dye colour was estimated quantitatively by calorimetric procedure using the UV-VIS Spectrophotometer (Bechman, USA) at optimum wavelength (λ_{max}) of 617 nm. Sample from the reactor was centrifuged (3000 g, 28°C) and the supernatant was assayed for the residual dye colour concentration. The performance of the reactor was also assessed by monitoring COD, pH, ORP, colour, SVI, VSS, SS, oxygen consumption rate (OCR) and dissolved oxygen (DO) throughout the reactor operation. The analytical procedures for estimating the above parameters were adopted from the procedures outlined in the standard methods [24].

Results and Discussion

After inoculation the reactor has taken 22 days (i.e. 22 cycles) for initial startup.

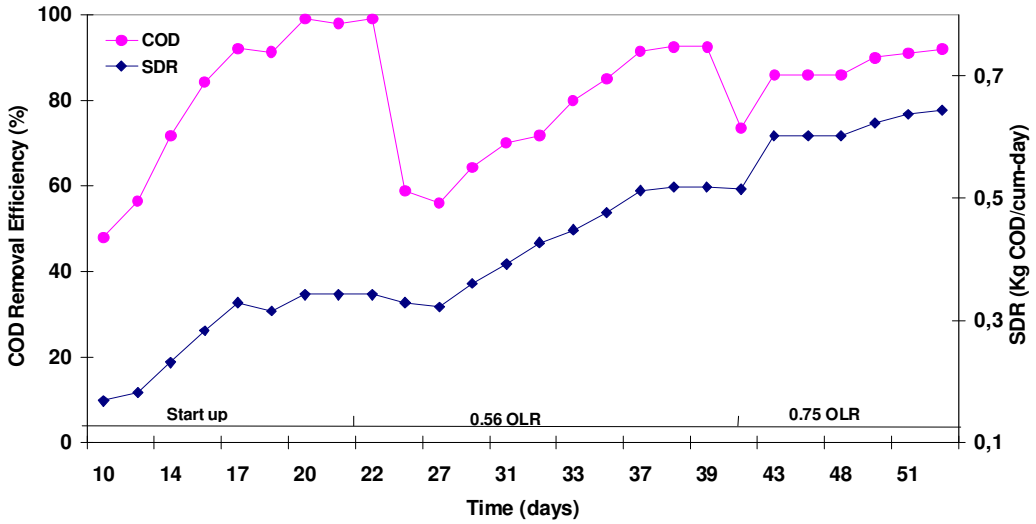

Figure 1. *COD and SDR variation during reactor operation*

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During startup phase reactor was operated with the designed synthetic feed (glucose as sole carbon source) at an OLR of 0.35 Kg COD/cum-day. On the whole the reactor was operated for a total number of 53 cycles (53 days) including startup period. After 17 days of startup operation a consistent performance with respect to COD removal was observed. Substrate degradation rate (SDR) profile also revealed a consistent substrate uptake rate (0.329 Kg COD/cum-day) after 17 days of inoculation.

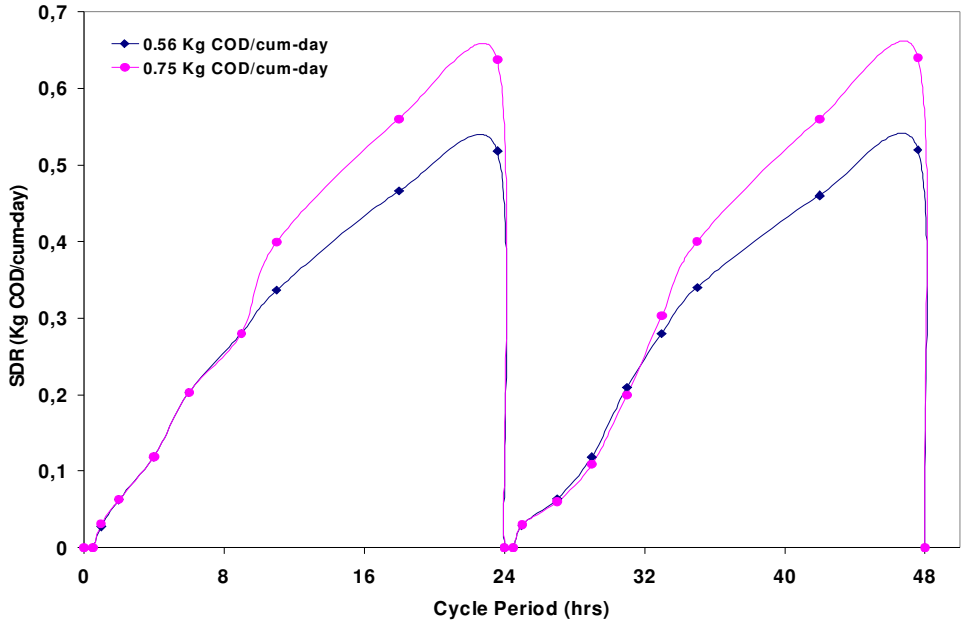

Figure 2. *SDR variation during two consecutive cycle operation*

On 23rd day of startup the reactor was fed with synthetic azo dye wastewater (OLR-0.56 Kg COD/cum-d). COD removal efficiency of 91% was observed after 37th day of operation (15 days after dye wastewater feeding) (Fig. 1). After achieving stable performance, the reactor was further operated with higher OLR (0.75 Kg COD/cum-d), where the reactor yielded 89% of COD removal efficiency after 39 days of startup (6 days after OLR shift). With continued operation, the reactor showed enhanced performance with respect to COD removal efficiency and attained stable conditions rapidly. The SDR profiles are consistent and comparable with the COD removal profiles for all the studied cases. SDR of 0.52 Kg COD/cum-d was observed at 0.56 Kg COD/cum-d of OLR at stable operating conditions, while with 0.75 Kg COD/cum-d of OLR, the reactor showed maximum SDR of 0.64 Kg COD/cum-d. The dye color (OD-617 nm) was also monitored during the reactor operation.

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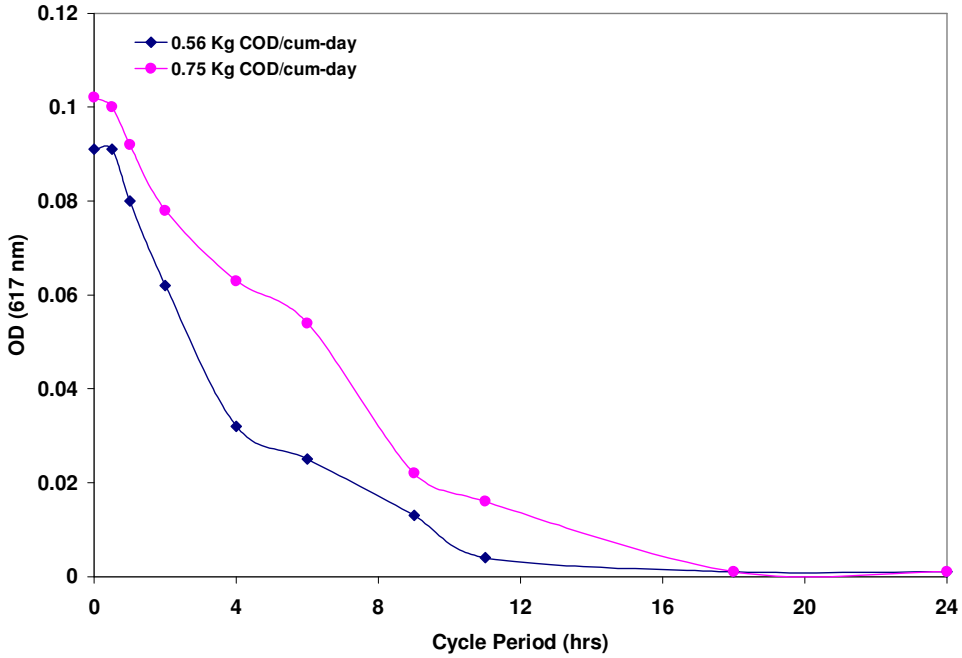

Figure 3. *Color removal variation during SBR cycle operation*

The colour removal was found to be initially rapid and approached a maximum after 10 hours (0.56 Kg COD/cum-d) and 18 hours (6 days for 0.75 Kg COD/cum-d) of cycle period. The COD removal rate was uniform throughout the reactor operation for the both OLRs studied and attained equilibrium after 20 h of the cycle operation. For achieving stable performance, the reactor showed to require 15 days at operating OLR of 0.56 Kg COD/cum-d and 6 days at operating OLR of 0.75 Kg COD/cum-d. Consolidated data of SBR performance at OLRs studied are presented in Table 2.

Table 2. *Performance of SBR at various OLRs studied*

OLR (Kg COD/cum-day)	**COD removal efficiency (%)**	**Colour removal Efficiency (%)**	**Time to achieve stable performance (days)**	**F/M ratio (as COD) range**
0.35 (startup phase)	92±1.4	-	22	0.129 - 0.096
0.56	92±1.9	100	15	0.179 – 0.150
0.75	92±1.2	100	6	0.197 – 0.187

The influent pH of 6.9 was introduced during the feeding and with the exhaustion of cycle period, the aqueous phase pH showed a gradual increase and approached 8.0 at the end of the reaction phase.

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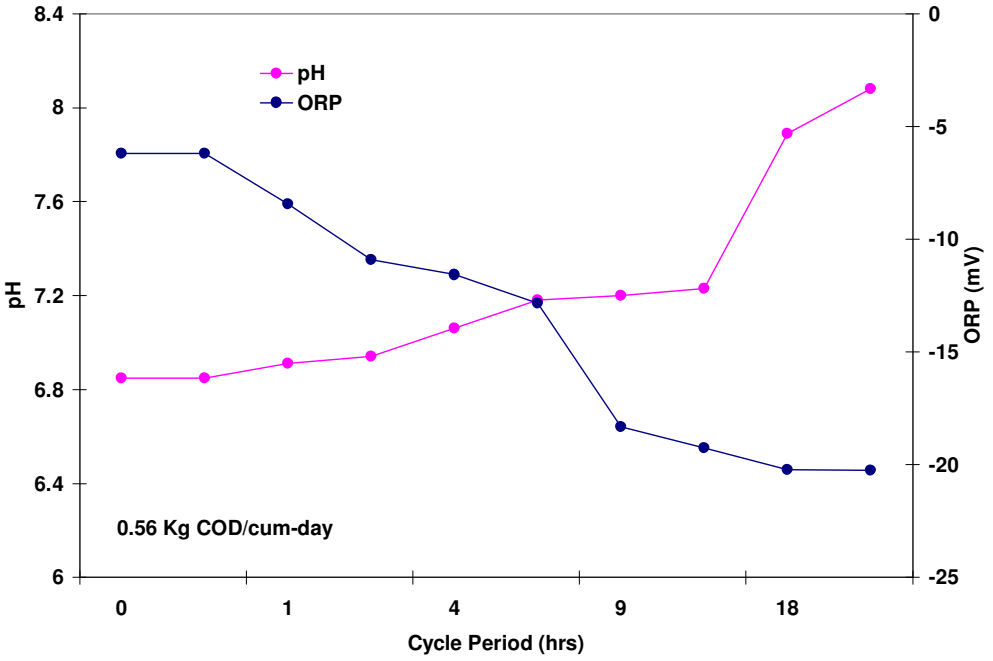

Figure 4. *Variation of pH and ORP during SBR cycle operation.*

ORP (mV) profile visualized a mirror image to pH. Oxygen consumption/transfer capacity often is one of the important factors that limit the capacity of suspended growth biological systems. Oxygen consumption rate (OCR) showed a rapid increase (0.098 mg O_2/min) during the initial phase of the cycle operation (upto 4 hours) for both the OLRs studied.

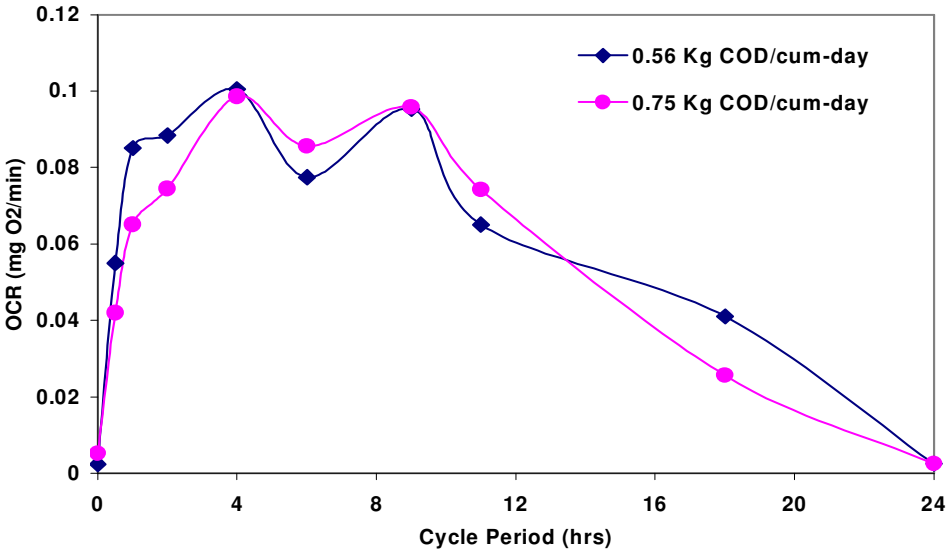

Figure 5. *OCR profile during SBR cycle operation.*

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Subsequently, the OCR showed a gradual reduction in the values. OCR is expected to increase with sludge retention time due to higher endogenous respiration requirements. Sludge volume index (SVI) was monitored during the end of each cycle operation before the decant phase. Initially at the startup phase, SV was 150 ml (0.56 kg COD/cum-d) and with increase in OLR also SV values showed similar values (45.9 ml). This may be attributed to the fact that increases in OLR relatively negligible inhibition in the process operation and its metabolic activities. The SVI was found to be good throughout the reactor operation (180 at 0.56 kg COD/cum-d and 198 at 0.75 kg COD/cum-d), which is less than 100. SVI of 100 is commonly used as the limit between good and poor settling sludge. It is reported that the sludge settles well and at high concentration of DO in periodically operated suspended growth systems produces sludge with good settling and thickening properties (floc high compactness) [21,25]. In this study aqueous phase DO was maintained consistently in the range of 2.5 to 3.0 mg/l and sludge showed good settling properties. F/M ratio was in the range of 0.129 to 0.096 during the startup phase of the reactor (Table 2). The F/M ratio showed more or less similar values for the two OLRs studied (0.179–0.150 at 0.75 kg COD/cum-d and 0.197–0.187 at 0.56 kg COD/cum-d), which indicates non-inhibition of process due to high dye substrate loading conditions. SS concentration varied in the narrow range of 4200 mg/l to 4500 mg/l and VSS concentration varied in between 2500 mg/l to 3200 mg/l during the reactor operation.

VSS/SS ratio was 0.65 initially during start up phase and with increase in cycle period the ratio showed a consistent increase and the viability of biomass increased with the increase in operating OLR (VSS/SS ratio of 0.74 [0.75 Kg COD/cum-d]).

Normally in the traditional aerobic wastewater treatment systems relatively less COD reduction is observed for dye based wastewater [1, 2, 26] while anaerobic processes demonstrated efficiency in reducing the colour of the wastewater [27-29]. Studies showed that anaerobic processes can be used to decolorize dye wastewater which improve the wastewater biodegradability for subsequent aerobic treatment [1, 3, 30-32]. Under anaerobic conditions, fermentative bacteria can reduce the dyes by cleaving the azo bond. It is a prerequisite to have anaerobic conditions to initially breakdown the dye molecule prior to the aerobic mineralization for effective treatment. To overcome the problem of the relative recalcitrance of azo dye breakdown products under anaerobic conditions, a two-stage treatment process was reported [33, 34]. In the first anaerobic stage, the azo dye was readily reduced to the corresponding colourless aromatic amines, which are then metabolized easily under aerobic conditions [2]. However in conventional biological process, the aerobic process is seldom capable of degrading the dye molecule while anaerobic process alone cannot handle the complete mineralization of the dye molecule. On the contrary, the SBR operation facilitates the inclusion of anoxic phase in addition to the aerobic phase in a single reactor during the sequence phase operation. In this study during feed phase (30 min) and subsequent settle and decant phase (30 min) in the cycle operation anoxic conditions persist in the reactor microenvironment. The anoxic (settle, decant and fill) microenvironment facilitates the initial breakdown of dye molecule and subsequently the products formed will be further mineralized in the aerobic phase (react). Size of the biofilm floc also has significant influence on the extent and presence of the anoxic zone [21]. Normally biofilm particle size in ASP is in the range of 10-110 µm. In the present study the suspended biofilm size in the reactor was in the range of 90-600 µm (average size 340 µm) and it is reported that the biofilm floc of 200 µm size and above will have anoxic microniches in

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the internal part of the flocs [35]. Venkata Mohan et al [21] reported sulphate reduction in SBR reactor configured with suspended growth due to induced anoxic environment. Effective dye materialization in the periodic discontinuous process may be attributed to the induced anoxic zone during cycle operation along with the anoxic environment prevailing in the suspended biomass. Time varying individual components of incoming wastewater in each process steps in periodic discontinuous operation facilitates microorganisms to be placed under nutritional changes from feast to famine state and it is also reported that periodic operations with altering feast and famine conditions results in high uptake of substrate and also better setability of the biomass [36]. Grau et al [37] in their accumulation regeneration theory stated that the more the microorganisms are able to store substrate during imposed transient phase, subsequent reuse for growth during substrate limited conditions have competitive advantage. Recent research indicated that the sequential feast and famine conditions enhance the overall performance of the biological system [38] especially for complex chemical wastewater treatment [21, 23].

Conclusions

It can be concluded from this experiments that the dye containing wastewater can be efficiently treated using periodic discontinuous operation. The induced anoxic conditions prevailing in the reactor due to the sequential batch operation facilitated initial azo reduction, which on subsequent aerobic process resulted in dye minerilization. The reactor performance was not inhibited at the studied two organic loading rates. The rapid startup and stabilization showed one of the important advantages of the SBR. Enforced short-term unsteady state conditions coupled with periodic exposure of the microorganisms to defined process conditions in SBR operation resulted in efficient performance for the treatment of dye based wastewater. Unlike the continuos biological operations the SBR process facilitates each individual operation to be carried out in the single reactor, which is considered to be flexible and moreover economical.

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EFFECT OF TWEEN 80 AND MOISTURE REGIMES ON ENDOSULFAN DEGRADATION BY *PSEUDOMONAS AERUGINOSA*

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Abstract. The problem of endosulfan bioremediation is poor solubility and restriction of appropriate biocatalyst. One promising approach in increasing the bioavailability of this organic compound is addition of surfactants. The synthetic surfactant Tween 80 was non-toxic to soil microorganisms and inert to the soil matrix and had the additional benefit of causing an enhanced dissolution rate for single compounds. The degradation of alpha and beta endosulfan by Pseudomonas aeruginosa with Tween 80 and different moisture regimes (flooded and non-flooded conditions) was studied. The rate of degradation was maximum (92 %) in non-flooded and Tween 80 added soil; the bacterial count was also maximum. The addition of synthetic surfactant Tween 80 enhanced the solubility and degradation of endosulfan. The degradation of both the isomers were observed and accompanied with formation of endodiol and endosulfan sulfate.

Keywords: *Tween 80; Moisture regimes; Endosulfan; Degradation; Pseudomonas aeruginosa*

Introduction

Endosulfan is a chlorinated cyclodiene insecticide currently used throughout the world for the control of numerous insects in a wide variety of food and non-food crops. Endosulfan has been ubiquitously detected in the atmosphere, soils, sediments, surface waters, rainwater's and foodstuffs [1]. Endosulfan comprises two-parent isomers alpha and beta endosulfan and the alpha to beta ratio of technical endosulfan is about 7:3 and both isomers are extremely toxic to aqueous organisms. Many bacteria and fungi including *Cornybacterium sp., Nocardia sp., Mycobacterium sp., Pseudomonas fluorescens, Penicillium sp., Aspergillus sp., Phanerochaete chrysosporium* have been reported to be endosulfan degraders [2]. Endosulfan could be degraded by attack on the sulfite group by oxidation and or hydrolysis to form the toxic endosulfan sulfate and the non-toxic endosulfan diol respectively [3]. The problem of endosulfan bioremediation is poor solubility and restriction of appropriate biocatalyst. One promising approach in increasing the bioavailability of this organic compound is addition of surfactants.

The synthetic surfactant Tween 80 was non-toxic to soil microorganisms and inert to the soil matrix and had the additional benefit of causing an enhanced dissolution rate for single compounds [4]. Despite the many advantages of surfactin over chemical surfactants for bioremediation, its use has been limited on account of the high production cost. The uptake of micellar n-decane and n-tetradecane was stimulated by a biodegradable synthetic surfactant resulting in higher growth rates. This was probably caused by direct uptake of the hydrocarbons along with the micelles [5]. The

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synthetic surfactant at low concentrations may be useful for bioremediation of sites contaminated with hydrophobic pollutants [6]. The addition of Tween 80 stimulated utilization of hexadecane by several strains of *Pseudomonas aeruginosa* [7]. *Pseudomonas aeruginosa* when combined with Tween 80 effectively enhanced the solubility and degradation of phenanthrene and they also reported that Tween 80 is biodegradable [8]. The oxygen uptake rate of bacterial cells was not affected in the presence of surfactants. They also reported that surfactant concentrations up to 10g/L could be used without any toxicity effect on microorganisms [9].

The environmental fate of endosulfan in soils is influenced by the pH, texture, moisture content and also by the presence of organic matter and co-pollutants. The rate of degradation is a function of the prevailing temperature, moisture regime, the content, type of organic matter and clays present [10]. The biodegradation of hydrophobic substances was affected by soil moisture, pH, mineral nutrients, micronutrients, organic supplements, treatment rate, treatment frequency and incubation temperature [11].

In the present study we have isolated an endosulfan degrader, *Pseudomonas aeruginosa* through intensive screening from endosulfan polluted soil samples. In addition, Tween 80 was added to the bacterial culture at different moisture regimes (Flooded and non-flooded) conditions to enhance the bioavailability and complete elimination of endosulfan from the contaminated soil.

Materials and Methods

Chemicals

Endosulfan standards (α-endosulfan, β-endosulfan and its metabolites) were purchased from M/s. Chem Service Inc., West Chester, USA with a purity of 98%. A standard was prepared by dilution of the stock solution (1000 µg/mL) of each compound and stored in a refrigerator at 4°C. All stock solutions were prepared and stored at -20°C. Commercial endosulfan was purchased from Jayaprakash Fertilizers Agency, Thiruvallur district. The non-ionic surfactant Tween 80 and 2-phenoxy ethanol were obtained from Sigma Chemical Co., USA. All other chemicals, solvents and reagents used in the study were of analytical grade.

Enrichment and isolation of bacterial strain

Two grams of soil samples was taken in a 250-mL Erlenmeyer flask containing 50 mL of liquid mineral medium (Ammonium chloride- 1.0g, Potassium dihydrogen orthophosphate-2.0g, Dipotassium hydrogen orthophosphate - 7.5g, Magnesium sulphate - 0.2g, Sodium chloride - 0.5g, Calcium carbonate - 0.2g, Glucose -1.0g, Distilled water - 1.0L, pH- 7.8 and agar 15% was added to solid medium) with 50µg/mL of endosulfan and was incubated for 7 days in a rotary shaker (IKA, Germany) at 130 rpm. Five millilitres of culture broth from individual flask culture was re-inoculated to 50 mL of endosulfan mineral salt medium and further cultured at 30˚C for 7 days. Enrichment of the culture was done by repeated transfers. Then 0.1 mL of culture broth was plated on solid endosulfan mineral salt medium for isolating single colonies. The single colonies were characterized and identified as a

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Pseudomonas aeruginosa using biochemical tests (*Table 1.*). The culture was maintained on mineral agar slants.

Table 1. *Biochemical test for Pseudomonas aeruginosa*

TEST	**RESULTS**
INDOLE	NEGATIVE
CITRATE UTILIZATION	UTILISED
T. S. IRON AGAR	K / K
FERMENTATIVE / OXIDATIVE (HUGH LEIFSON)	OXIDATIVE FERMENTATION
OXIDASE	POSITIVE

Growth studies

A loopful of ***P.aeruginosa*** grown on mineral agar slant was inoculated into a 250 mL conical flasks containinig 50 mL medium amended with endosulfan as substrate. The flasks were kept for 24 h on an orbital rotary shaker set at 130 rpm at 30°C for the growth of the organism. Growth was measured at 24 h as viable cell count as well as turbidity at 550 nm. The cells were harvested by centrifugation at 5000 g for 10 min. The cell pellet was washed twice in 15 mM phosphate buffer, pH 7. The washed cells were used for degradation study ($2x10^5$ CFU).

Soil

The soil selected for this study was collected from Thiruvallur district had no previous exposure to pesticides and was classified as sandy loam. The soil was air dried and sieved through 2mm sieve. The carbon content of the soil was estimated using TOC analyzer (Analytic gena, micro C). The pH of the sample was estimated as per the methods of [12]. pH of the soil was 7.3, organic carbon 0.12%. Commercial endosulfan 35EC was added to the soil to give a concentration of 2.35 mg/g active ingredient (compound that kills or controls the target pest). After air drying for 24 - 48 h, the soil was pulverized and used for degradation studies.

Degradation of endosulfan in flooded and nonflooded condition

For each moisture regimes, 4 sets were made containing 20g soil in each set's and Tween 80 was added at CMC (A phenomenon unique to surfactants is the self-assembly of molecules into dynamic clusters called micelles. Micelle formation occurs above a critical concentration of surfactant monomers referred to as the Critical Micelle Concentration [13]. Three replicates were maintained for each set. Experimental details were presented in *Table 2*. All the experimental sets were received 6 mL of mineral medium without glucose. To maintain the flooded condition, 20 mL of mineral medium was added as an addition. All sets were kept at 30°C for 8 weeks and 1.2g samples were taken at weekly intervals to quantify the residual endosulfan present in each soil sample.

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Table 2. *Experimetal set up*

T1	Soil (flooded) + Endosulfan
T2	Soil (flooded) + Endosulfan + *Pseudomonas aeruginosa*
T3	Soil (non-flooded) + Endosulfan
T4	Soil (non-flooded) + Endosulfan + *Pseudomonas aeruginosa*
T5	Soil (flooded) + Endosulfan + Tween 80 (0.1 g/L)
T6	Soil (flooded) + Endosulfan + Tween 80 (0.1 g/L) + *Pseudomonas aeruginosa*
T7	Soil (non-flooded) + Endosulfan + Tween 80 (0.1 g/L)
T8	Soil (non-flooded) + Endosulfan + Tween 80 (0.1 g/L) + *Pseudomonas aeruginosa*

Bacterial count

During the degradation process, the bacterial population (plate count technique) was monitored at weekly intervals. One gram of soil was taken and incubated for 30 min with 100 mL of sterile water at 30°C at 150 rpm. A 100 µL sample of appropriate dilution of the soil suspension was inoculated onto nutrient agar plates. The plates were incubated at 30°C for 24 h and the number of colonies were counted.

Extraction of endosulfan residues

In the experiment with flooded and non-flooded soils, the volumes in all the bottles were raised to 25 mL with mineral medium before harvesting. An equal volume of ethyl acetate was added and samples were shaken on an orbital shaker at 220 rpm for 30 minutes. Contents were then transferred to a separating funnel and the organic layer was collected. The aqueous layer was extracted three more times with 25 mL of ethyl acetate. Ethyl acetate fractions were pooled, passed through anhydrous sodium sulfate (5g) and florosil (2g) mixture [14] and evaporated at room temperature.

Chromatographic analysis

Pesticide residue was dissolved in acetone and an aliquot containing 5-10 µg of endosulfan was spotted on a silica gel plate and the chromatogram was developed in hexane: chloroform: acetone (9:3:1). The separated spots were visualized by spraying the chromogenic reagent ($AgNO_3$ in 2-phenoxy ethanol) [15]. For gas chromatography, the residual pesticide was dissolved in 1 mL of acetone, diluted to 10^6 times with n-hexane, and analysed as per the above said procedure.

One microlitre from each of the final residue solutions was injected into the GC (Chemito) model 1000 chromatography equipped with a packed glass column (4'x1/8", filled with 60-80 mesh coated over chromosorb with a mixture of 1.5% OV-17 and 1.95% QF) with ECD (Electron Capture Detector) for residue analysis in an injector temperature of 220°C, oven temperature 190°C, detector temperature 280°C and nitrogen as carrier gas (27 mL/min). The qualitative identification of the endosulfan present in the samples was performed by comparing the relative retention times (RRT) with respect to the standard, for each peak in the real sample chromotogram to those (RRT) in the standard mixture chromotogram. The quantitative determination was performed by using the relative peak areas (RPA) and the relative concentrations (RC).

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Results and Discussion

Effect of flooded and non-flooded condition on endosulfan degradation

Treatment unit T2 at flooded condition showed 40% of alpha and 29% beta degradation, the bacterial population was 53 x 10^6 CFU/g of soil. The control unit T1 recorded 10% of alpha and 8% of beta degradation; the bacterial population was 63 x 10^4 CFU/g of soil (*Fig. 1.*).

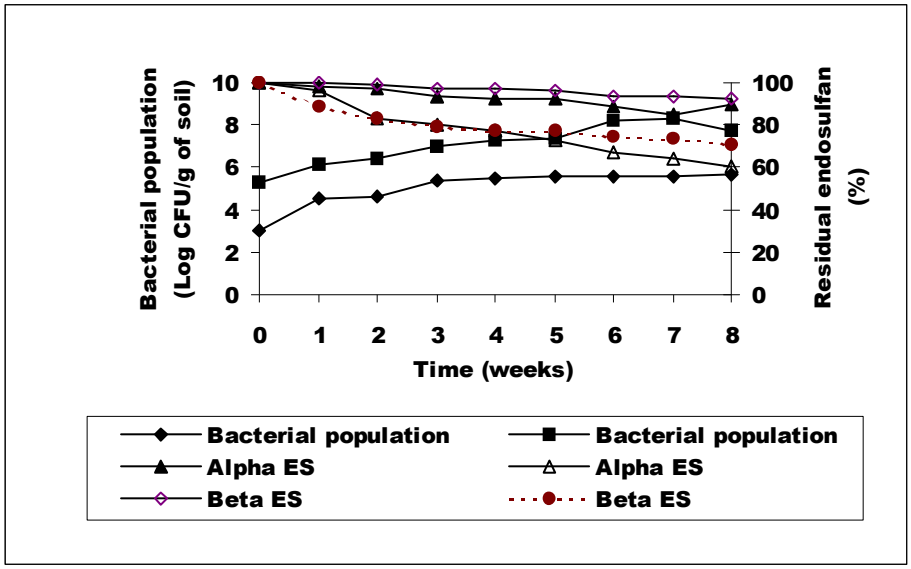

Figure 1. *Degradation of endosulfan (ES) at flooded condition*

The unit T4 inoculated at non-flooded condition showed 82 % alpha and 60 % beta endosulfan degradation, the bacterial population was 62 x 10^8 CFU / g of soil. The control unit T3 showed 14 % of alpha endosulfan and 10% of beta endosulfan degradation where the bacterial population was 28 x 10^4 CFU/g of soil (*Fig.2*).

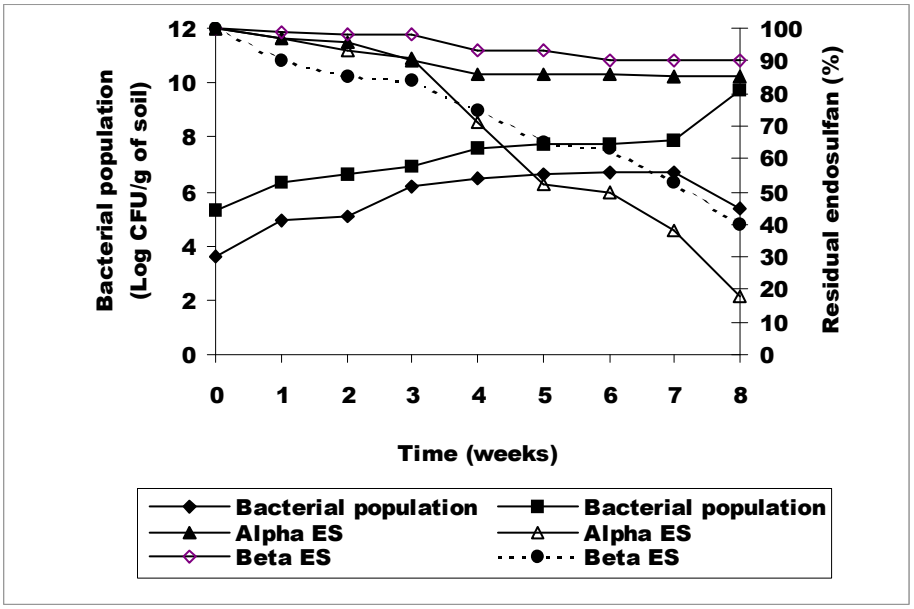

Figure 2. *Degradation of endosulfan (ES) at non- flooded condition*

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During the degradation process, the metabolites such as endosulfan diol and endosulfan sulfate were formed at flooded and non-flooded conditions (*Fig. 3*).

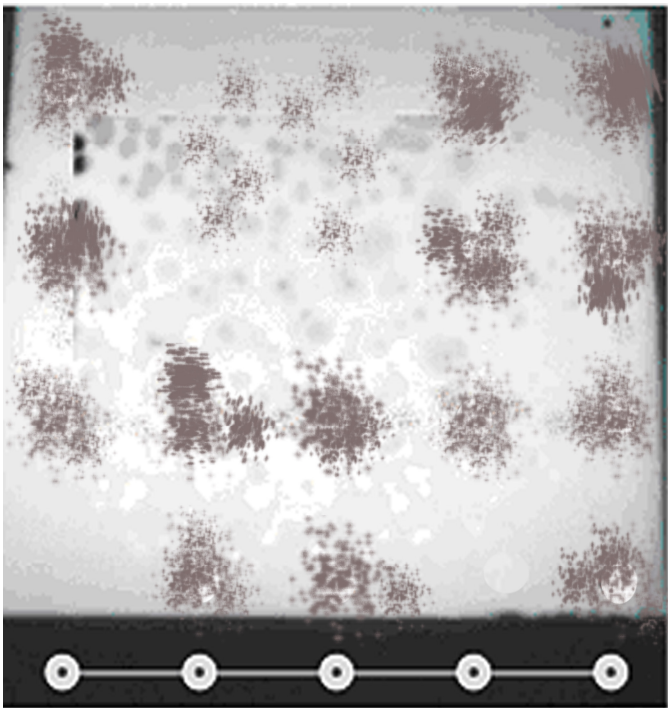

Figure 3. *Thin layer chromatogram showing the metabolites formed during the degradation of α and β endosulfan at flooded (F) and non-flooded (NF) condition*
(1) α- endosulfan, (2) β-endosulfan, (3) Endosulfan sulfate
(4) Endodiol
(a) without Pseudomonas aeruginosa
(b) with Pseudomonas aeruginosa

The maximum degradation at non-flooded condition is mainly due to increased growth and activity of the bacteria in non-flooded condition [16]. This could be due to better bioavailability of endosulfan and optimal biotic activity of cells at this condition.

Effect of Tween 80 on endosulfan degradation

Microbial methods to remediate hydrophobic organochlorines contaminated soils are often limited by low substrate solubilities which can reduce bioavailability to the degrading microorganisms [17]. Use of Tween 80 as a means of increased bioavailability of hydrophobic endosulfan to microorganisms under different moisture regimes (flooded and non-flooded conditions) were studied.

The unit T6 (flooded with Tween 80) recorded 48% of alpha and 38% of beta endosulfan degradation. The bacterial population in the soil was 36 x 10^8 CFU/g of soil. The control unit (T5) showed 10% of alpha endosulfan and 6 % of beta

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endosulfan degradation, the bacterial population in the soil was 5 x 10^5 CFU/g of soil (*Fig. 4.*).

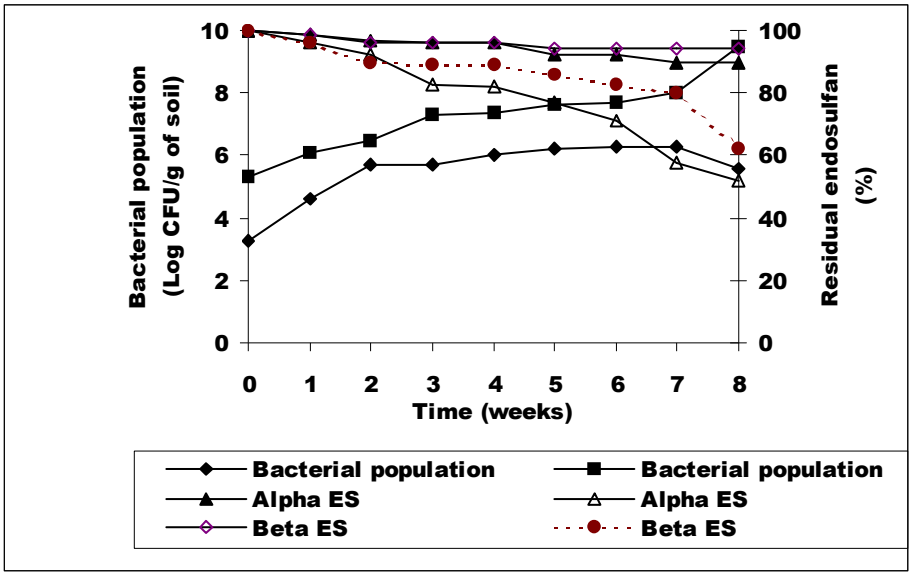

Figure 4. *Degradation of endosulfan (ES) with Tween 80 at flooded condition*

When compared to Tween 80 with flooded and non-flooded conditions, Tween 80 with non-flooded conditions recorded maximum degradation of endosulfan isomers (92% of alpha and 87% of beta), the bacterial population in the treatment unit T8 was 95 x 10^9 CFU/g of soil. The control unit (T7) showed 13% of alpha and 10% of beta endosulfan degradation. The cell growth was increased to 3 x 10^6 CFU/g of soil (*Fig.5.)*

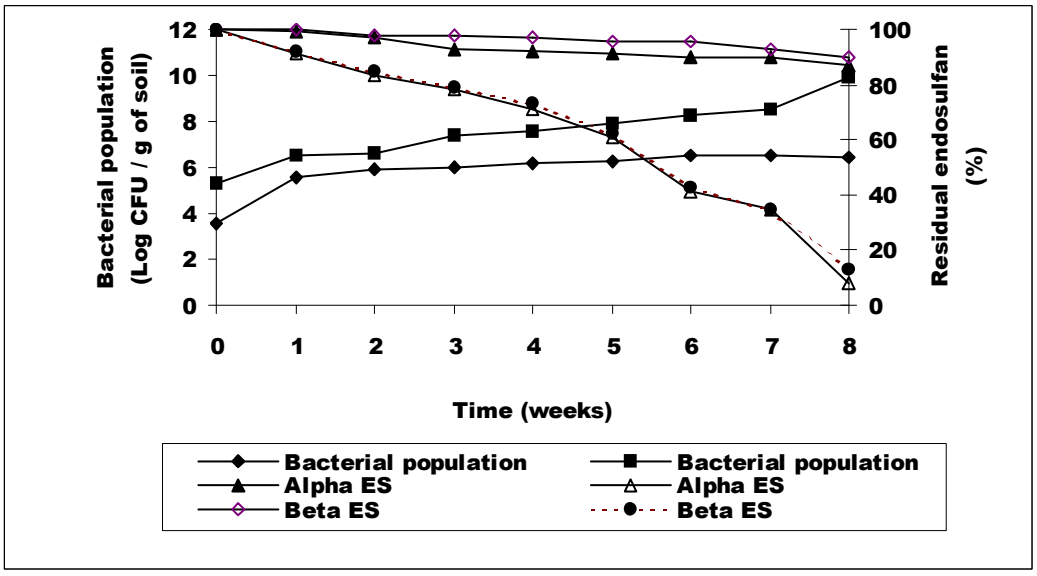

Figure 5. *Degradation of endosulfan (ES) with Tween 80 at non-flooded condition*

Thin layer chromatography results of endosulfan diol and endosulfan sulfate formed during the degradation process are given in the *Fig. 6.*

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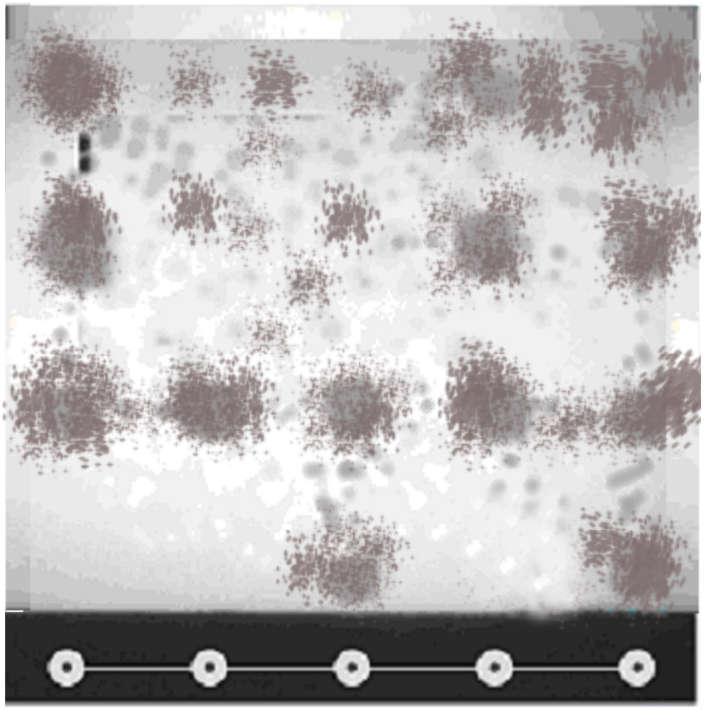

Figure 6. *Thin layer chromatogram showing the metabolites formed during the degradation of α and β endosulfan at flooded (F) and non-flooded (NF) condition with Tween 80*
(1) α- endosulfan, (2) β-endosulfan, (3) Endosulfan sulfate
(4) Endodiol
(a) without Pseudomonas aeruginosa
(b) with Pseudomonas aeruginosa

The unit T8 (*Pseudomonas aeruginosa* with Tween 80), showed optimum degradation of endosulfan isomers. The addition of Tween 80 (0.1g/L) in the treatment unit T8 might have emulsified the endosulfan, thereby increasing the amount of insecticide in contact with the soil bacteria. Surfactant even at very low concentration was shown to enhance the biodegradation of certain xenobiotics in soil [18]. The nonionic surfactant (Novel 1111412-56) at 10 µg/mL added to the surface of lime silt loam soil enhanced the biodegradation of phenanthrene and biphenyl [6].

The accumulation of metabolite endosulfan sulfate is more persistent and is similarly toxic as the alpha and beta isomers of endosulfan. Under alkaline conditions the transformation of endosulfan-to-endosulfan diol was observed which was reported to be less toxic than endosulfan sulfate. These observations are in accordance with an earlier study, where an enriched bacterial culture (*Mycobacterium sp*) was shown to metabolize the two isomers of endosulfan differentially. Alpha-isomer was subjected to the oxidation and converted into endosulfan hydroxy ether, endosulfan diol and some uncharacterized metabolites. Beta endosulfan appears to be much more persistent in soil than the alpha-isomers under flooded and non-flooded condition [19]. A number of studies carried out on the degradation of endosulfan in aqueous system, where beta endosulfan is degraded faster than the alpha isomers [20]. The degradation of soil

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bound alpha endosulfan is always faster than beta-isomers under both aerobic and anaerobic conditions [21, 22]. The formation of endosulfan sulfate was quite high in non-flooded experiments [23, 24]. In uninoculated soils, there is a formation of endosulfan sulfate. This indicates that living organisms may be necessary to bring about the oxidation of endosulfan-to-endosulfan sulfate [25]. The degradation of soil bound endosulfan was slow; the reason may be due to the adsorption of endosulfan to soil particles or because of the presence of other carbonaceous materials in the soil [26].

Most of the research has concentrated on the degradation of endosulfan in liquid medium [19]. A few studies carried out on the degradation of endosulfan in soil have been without surfactant amendment [26]. This study proves that *Pseudomonas aeruginosa* combined with Tween 80 is able to achieve 92% degradation of endosulfan in contaminated soil. Hence, surfactant enhanced degradation studies is a promising approach for remediation of endosulfan contaminated soils.

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KINETICS AND EQUILIBRIUM STUDIES ON THE BIOSORPTION OF HEXAVALENT CHROMIUM FROM AQUEOUS SOLUTIONS USING *BACILLUS SUBTILIS* BIOMASS

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Abstract. Heavy metal contamination of industrial effluents is one of the significant environmental problems due to their toxic nature and accumulation throughout the food chain as non-biodegradable pollutants. In this study, dead Bacillus subtilis biomass was assessed for its efficiency to remove chromium(VI) from aqueous solutions. Optimum pH and temperature for biosorption of Cr(VI) were found to be 2.0 and 30oC, respectively. The biomass has the maximum biosorption capacity of 14.54 mg/g of biomass at 100 ppm initial chromium concentration and 2 g/l biomass loading. The biosorption process followed pseudo first order kinetic model, implying that the initial rate of biosorption is totally independent of the initial concentration. The biosorption of Cr(VI) is well described by Langmuir isotherm, which express the existence of monolayer adsorption under the experimental conditions. The adsorption-desorption experiments performed inferred the reusability of the biomass. X-ray photoelectron spectroscopy studies revealed that chromium bound on to the B. subtilis biomass was in trivalent form.
Keywords: *Biosorption, Bacillus subtilis, hexavalent chromium, adsorption isotherms, biomass*

Introduction

Rapid industrialization has led to increased disposal of heavy metals and radionuclides into the environment. Current industrial metal effluent treatment methods posses various disadvantages such as lack of cost effectiveness, production of toxic chemical sludge, etc. Therefore the removal of toxic heavy metals to an environmentally safe level in a cost effective and environment friendly manner assumes greater importance. Biosorption, an environment friendly technology to clean up the environment based on the utilization of dead biomass can be an efficient and cost effective remedy.

Chromium is a toxic metal of widespread industrial use and exists in several oxidation states. The most stable and common forms are the trivalent Cr(III) and the hexavalent Cr(VI) species, which display quite different chemical properties. Chromium(VI) is designated and widely recognized to be a human inhalation carcinogen [1]. Chromium(III) is less toxic when compared to Chromium(VI) and it has low acute and chronic toxicity to humans at high doses. High doses of Chromium(VI) compounds are also associated with nephrotoxicity [2, 3]. Acute exposure to high levels of Chromium(VI) can produce nervous system damage and liver disorder [1, 3].

Extensive use of chromium in electroplating, tanning, textile dyeing results in the effluents containing Cr(VI) and Cr(III) at concentrations ranging from tenths to hundreds of milligrams/litre. Several procedures have been proposed for removal of chromium(VI) from industrial wastewaters. Conventional methods for removing chromium(VI) ions from wastewater include: chemical reduction, membrane separation, electrochemical

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treatment, ion exchange and evaporative recovery. Although the effectiveness of these methods has been proved, they suffer from a major disadvantage, namely lack of cost effectiveness. Other limitations include energy intensive processing and concentration dependence, low efficiency, not feasible to reduce the chromium concentration to levels as low as required by environmental legislation and production of toxic chemical sludge, which needs additional treatment. These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1–100 mg/l [4]. Hence many researchers worked on the biosorption of Cr(VI) using different biosorbents such as *Rhizopus*, dead fungal biomass of *Aspergillus niger, Rhizopus oryzae*, *Saccharomyces cerevisiae, Ecklonia* biomass, peat moss and modified saw [5-9].

Bacillus subtilis is a Gram-positive, aerobic, rod-shaped bacterium and ubiquitous in soils and waters. Its parietal structure is well known and is composed primarily of peptidoglycan and teichoic acid [10]. Peptidoglycan is a polymer of acetylglucosamine and acetylmuramic acid, which carry mainly carboxylic and hydroxyl functional groups. On the other hand, teichoic acid is a polymer of copyranosyl glycerol phosphate, which carries mostly phosphate and hydroxyl groups. *B. subtilis* is widely used in the commercial production of various enzymes. In this study, dead *B. subtilis* biomass has been used for the removal of hexavalent chromium. Advantages of using dead *B. subtilis* biomass mainly recline on the abundant availability of the source of biomass, which comes from the existing enzyme fermentation industries. Also, the use of dead cells in biosorption is most advantageous for wastewater treatment, in that, the dead organisms are not affected by toxic wastes; they do not require a continuous supply of nutrients and they can be regenerated and reused for many cycles [11]. Dead cells may also be stored or used for extended periods at room temperature without putrefaction.

Materials and methods

Preparation of biomass

Bacillus subtilis strain was obtained from Tamil Nadu Agricultural University, Coimbatore. It was grown and maintained on both nutrient broth and nutrient agar. It was cultivated at room temperature in medium containing: soluble starch-20 g/l; beef extract-10 g/l; yeast extract -2 g/l; peptone -5 g/l; NaCl - 5 g/l. (pH adjusted to 7.2). After a week of incubation at 33±1°C, biomass was harvested by means of centrifugation at 10,000 rpm for 10 minutes, washed twice with distilled water and then dried for 6 hours at 80°C in an air oven. The dried biomass was then crushed with a mortar and pestle to a fine powder. The powdered biomass was stored in an air tight pack and used for biosorption.

Chemicals

$K_2Cr_2O_7$ used in the study was of analytical grade procured from Ranbaxy Chemicals. All other reagents used were of analytical grade, unless stated otherwise. Double distilled water has been used throughout the project work, unless stated otherwise. Chromium solution of different concentrations was prepared by suitably diluting the 1000 ppm stock solution to known volumes.

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Estimation of chromium

Amount of chromium in a given solution was determined spectrophotometrically at 540 nm using 1, 5-diphenyl carbazide as the complexing agent [12]. The sample containing Cr(VI) ions was mixed with 1 ml of 3 N H_2SO_4 and 1 ml of 0.25% 1, 5-diphenyl carbazide solution and made up to known volume. The absorbance at 540 nm was measured for the purple coloured solution after 10 minutes ageing. A calibration curve was drawn in the range of 5 to 50 ppm by plotting absorbance against concentration of chromium.

Biosorption experiments

Batch biosorption experiments were conducted in 100 ml Erlenmeyer flasks containing 50 ml chromium solution. Equilibrium studies were performed using 100 mg dried ground biomass per 50 ml of chromium solution. The test solutions were agitated on shaker with temperature controller at a constant speed of 75 strokes per minute. Samples were taken at definite intervals (0, 10, 20, 30, 45, 60, 120, 180, 240, 300, 360, 420 and 480 min), centrifuged at 10,000 rpm for 5 min to remove the biomass and analyzed for residual metal ion concentrations. Chromium ions adsorbed on to the biosorbent was calculated from the difference between the metal ion concentration in the solution before and after the biosorption process.

In order to find out the effect of pH on the biosorption process and to find the optimum pH, equilibrium batch experiments were carried out at various pH ranging from 2 to 8 by keeping all the other parameters constant. The batch biosorption experiments were also done at different temperatures (25, 30, 35 and 40 ^{o}C) to find the optimum temperature for biosorption. To study the effect of initial chromium concentration on the equilibrium uptake by the biomass, batch experiments were done at various initial metal ion concentrations (50, 75, 100, 125 and 150 mg/l). In order to find out the effect of the biomass loading on the uptake of chromium, batch experiments were done at various biomass concentrations (1, 1.5, 2, 2.5 and 3 g/l).

The Cr(VI) uptake by *B. subtilis* biomass was calculated from the difference between the initial and final chromium concentration as follows,

$$q = \frac{(C_o - C_e)}{S} V \qquad \text{(Eq.1)}$$

where, q is the Cr(VI) uptake by the biomass (mg/g), C_o is the initial Cr(VI) concentration (mg/l), C_e is the final Cr(VI) concentration (mg/l), S is the biosorbent dosage (g) and V is the solution volume (l).

Desorption experiments

In order to determine the reusability of biosorbent consecutive adsorption-desorption cycles were repeated three times by using the same biosorbent. Desorption of Cr(VI) ions was performed by 0.1 M NaOH solution. Biomass loaded with Cr(VI) ions was placed in the desorption medium and agitated for a period of 3 hours at a constant speed of 75 strokes per minute. The final Cr(VI) concentration in the aqueous phase was determined by using a spectrophotometer. After each cycle of adsorption–desorption, biosorbent was

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washed with distilled water and reconditioned for adsorption in the succeeding cycle. The desorption ratio was calculated from the amount of metal ions adsorbed on the *B. subtilis* biomass and the final Cr(VI) ion concentration in the adsorption medium.

XPS analysis

The X-ray photoelectron spectroscopy (XPS) studies were carried out by a VG Microtech Multilab ESCA 3000 spectrometer with non-monochromotized MgKα X-ray source (hν = 1253.6 eV). The samples were degassed for several hours in the chamber to minimize air contamination to sample surfaces.

Results and discussion

Effect of pH

pH is a vital parameter affecting the biosorption process. The results obtained are shown in *(Fig. 1.)*

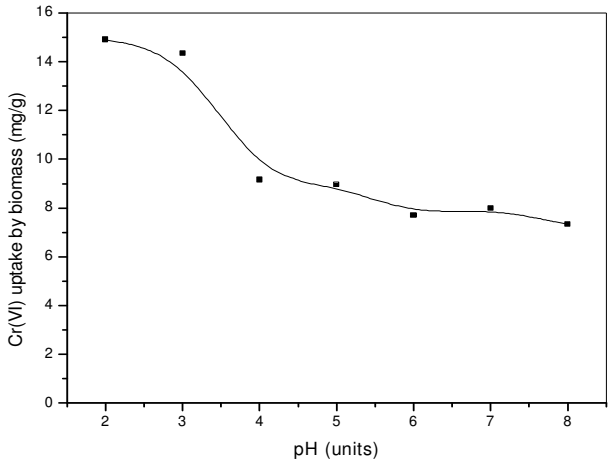

Figure 1. *Effect of pH on the Cr(VI) uptake by B. subtilis: Cr(VI) conc. 100ppm, biomass concentration=2g/l, Temperature=30^{o}C agitation=75 strokes per minute.*

From the figure, it is observed that the biosorption efficiency of Cr(VI) decreased as the pH increased. The maximum uptake capacity of the biomass was 14.9 mg/g for an initial Cr(VI) concentration of 100 ppm at pH of 2.0.

The increased binding of hexavalent chromium at low pH can be explained by two factors. First, adsorption of Cr(VI) at pH 2.0 suggests that the negatively charged chromium species (chromate/dichromate in the medium) bind through electrostatic attraction to positively charged functional groups on the surface of biosorbents. As the pH increased, the overall surface charge on the cells became negative and biosorption decreased. In alkali conditions, carboxylate group exists in deprotonated form and has net negative charge. As a result, the surface charge of the biosorbents become negative and biosorption of Cr(VI) decreases.

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Secondly, the solution chemistry of chromium(VI) ions can affect the biosorption process. Previous studies showed that chromium exhibits different types of pH dependent equilibria in solutions. Sorbate and chromium form stable complexes such as $Cr_2O_7^{2-}$, $HCrO_4^-$, CrO_4^{2-}, and $HCr_2O_7^-$, the fraction of any particular species is dependent on chromium concentration and pH. In low chromium concentration, the main fraction is $HCrO_4^-$ with pH below 5.0, whereas the CrO_4^{2-} increases with increase of pH value and becomes the main form with pH above 7.0 [13, 14].

Effect of temperature

In the present study, the temperature is varied from 25 to 40°C. With the increase of temperature from 30 to 40°C, the uptake decreases from 28.76 to 24.32%. The results obtained are illustrated in (*Fig. 2.*).

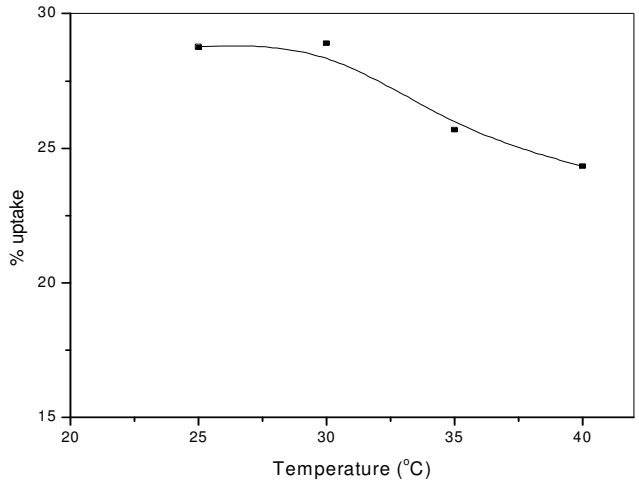

Figure 2. *Temperature versus % Cr(VI) uptake by B. subtilis: Cr(VI) conc. 100ppm, biomass concentration=2g/l, pH=2, agitation=75 strokes per minute.*

From the figure, it is observed that the uptake capacity is influenced by the experimental temperature conditions. The decrease of equilibrium uptake with the increase in temperature may be due to the exothermic nature of adsorption process.

Effect of initial metal concentration

Cr(VI) sorption was studied in batch experiments (pH 2.0) using different initial Cr(VI) concentrations of 50, 75, 100, 125, 150 ppm. The equilibrium uptake of the biomass was 12.03 mg/g (48.64%), 13.28 mg/g (35.57%), 14.34 mg/g (28.88%), and 14.73 mg/g (23.61%) and 15.02 mg/g (20.10%) at initial concentration 50, 75, 100, 125, and150 ppm chromium, respectively (*Fig. 3.*).

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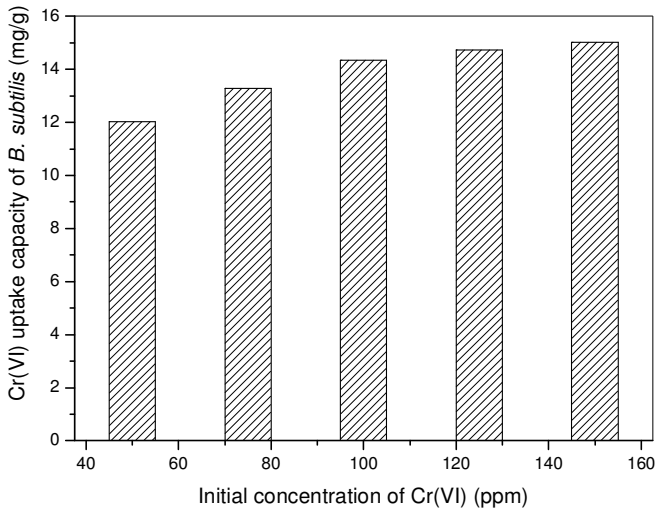

Figure 3. *Effect of initial concentration of Cr(VI) on uptake capacity: biomass concentration=2g/l, pH=2, Temperature=30°C,agitation=75 strokes per minute.*

It is evident that the amount of chromium adsorbed onto the biomass increases gradually with an increasing concentrations of Cr(VI). The increase of adsorption yield with the increase in metal ion concentration is probably due to higher interaction between the metal ions and metal sequestering sites of biosorbent.

Effect of biomass concentration

The Cr(VI) uptake by the biomass decreases with the increase in biomass concentration (*Fig. 4*).

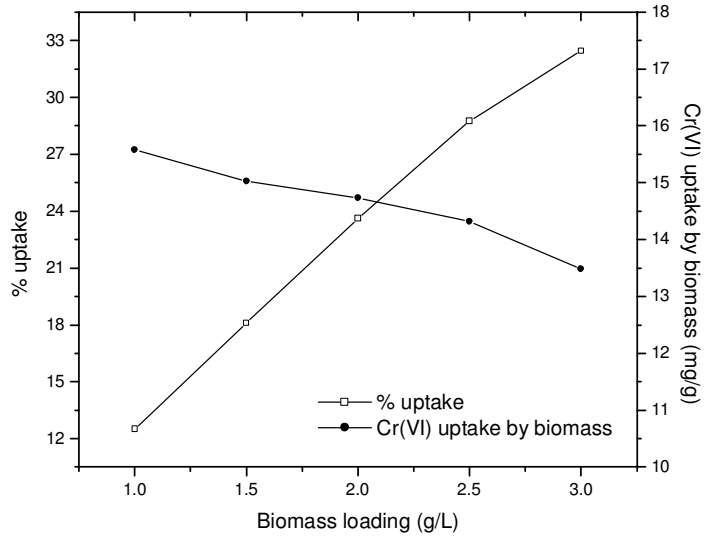

Figure 4. *Effect of biomass loading on % uptake and uptake capacity: Cr(VI) conc. 100ppm, pH=2, Temperature=30°C, agitation=75 strokes per minute.*

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When the biomass concentration increased from 1 to 3 g/l, the adsorption capacity of biomass decreased from 15.57 to 13.48 mg/g. The decrease in metal uptake by increasing the biosorbent dosage may be due to complex interactions of several factors. The important factor being at high sorbent dosages the available metal ions are insufficient to cover all the exchangeable sites on the biosorbent, usually resulting in low metal uptake. In gold biosorption by dried biomass of *Azolla filiculoides*, 5% decrease in gold uptake efficiency was observed when the biomass concentration was increased from 1 mg/l to 9 mg/l [15].

Biosorption kinetics

The initial adsorption rate was rapid and thereafter adsorption was gradual and equilibrium was reached after 8 hours. Kinetic models such as pseudo first order and pseudo second order have been used to describe the kinetics of adsorption. The rate constants of chromium adsorption on biomass were determined using the pseudo first order (Lagergren rate equation) expression shown below, [16]

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \qquad \text{(Eq.2)}$$

where, k_1 is the Lagergren rate constant and q_e and q_t are the amounts of chromium adsorbed (mg/g) at equilibrium and at time t, respectively. The straight-line plots of $\log(q_e-q_t)$ versus t for different chromium concentrations indicate the applicability of the above equation to chromium biosorption on the biomass. The values of k_1 and R^2 along with the calculated uptake capacity at particular initial concentration are provided in (*Table 1.*)

Table 1. *Rate constants of Cr(VI) biosorption by dead B. subtilis*

		First order rate constants			**Pseudo second order rate constants**			**Intraparticle diffusion rate constants**	
Initial conc. (m g/g)	**$q_{e\ (exp)}$ (mg/g)**	**k_1 (min^{-1})**	**$q_{e\ (cal)}$ (mg/g)**	**R^2**	**k_2, ($gmg^{-1}\ min^{-1}$)**	**$q_{e\ (cal)}$ (mg/g)**	**R^2**	**k_p ($mgg^{-1}min^{-1/2}$)**	**R^2**
50	12.04	4.61×10^{-03}	12.47	0.971	1.17×10^{-04}	21.42	0.831	0.64	0.984
75	13.30	4.25×10^{-03}	13.55	0.982	1.01×10^{-04}	23.66	0.823	0.69	0.984
100	14.40	4.12×10^{-03}	14.69	0.976	8.79×10^{-05}	26.04	0.796	0.75	0.982
125	14.80	4.27×10^{-03}	15.42	0.973	7.22×10^{-05}	28.51	0.79	0.78	0.981
150	15.20	4.28×10^{-03}	15.67	0.971	8.35×10^{-05}	27.56	0.784	0.80	0.981

Cr(VI) conc. range 50-150 ppm, pH=2, Temperature=30°C

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The kinetics of adsorption can also be described by pseudo second order equation and it is given by [17]

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad \text{(Eq.3)}$$

where k_2 (g/mg min) is the second order rate constant. The straight line plots of t/q_t versus t for different chromium concentrations indicate the applicability of the above equation to chromium biosorption on the biomass. The second order rate constant, R^2 along with the calculated uptake capacity at particular initial concentration are provided in (*Table 1*). From the table, it is very clear that the sorption of Cr(VI) by *B. subtilis* biomass follows pseudo first order kinetic model, implying that this system is totally independent of initial concentration.

Adsorption process incorporates the transport of adsorbate from bulk solution to the interior surface of the pores. In some adsorption processes this step becomes the rate-controlling factor. Hence, the data obtained were further processed for testing the role of diffusion (as the rate controlling step) in the adsorption process. The rate parameters for intraparticle diffusion (k_d) for Cr(VI) were determined by using the following equation,

$$q_t = k_d \sqrt{t} \qquad \text{(Eq.4)}$$

where, k_d is the rate constant of intraparticle diffusion parameter ($mgg^{-1}min^{-1/2}$).

According to the Weber and Moris model [18], uptake is proportional to the square root of contact time during the course of adsorption. (*Fig. 5.*) shows a plot of q_t versus $\sqrt{t}$ for the present system. It is known that if the intraparticle diffusion is the rate limiting step then the lines should pass through the origin. It can be seen from the figure that the lines didn't pass through the origin. Hence, in this case, intraparticle diffusion is not the rate-limiting step.

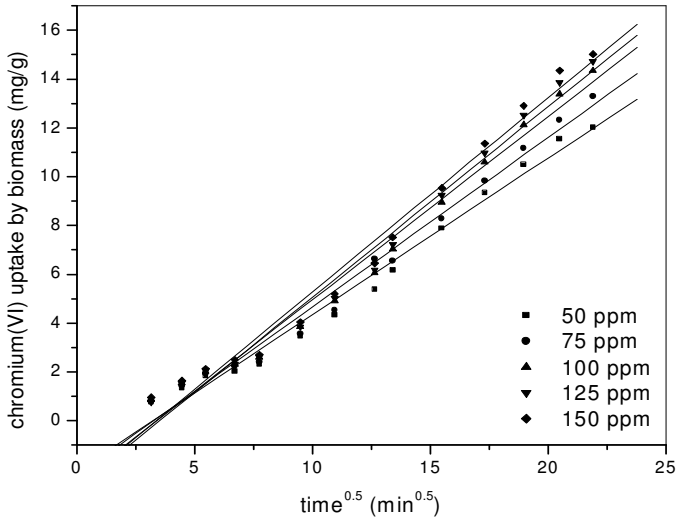

Figure 5. *Intraparticle diffusion model of Cr(VI) biosorption by dead B. subtilis*: *pH=2, Temperature=30^{o}C, adsorption period =8 hours.*

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Analysis of adsorption isotherms

Adsorption isotherms were used to characterize the interaction of each chromium species with the adsorbent. This provides a relationship between the concentration of Cr(VI) in the adsorption medium and the amount of Cr(VI) adsorbed on the solid phase when the two phases are at equilibrium.

Langmuir and Freundlich adsorption isotherms are the two widely used isotherms. The Langmuir model is based on the assumption of surface homogeneity such as equally available adsorption sites, monolayer surface coverage, and no interaction between adsorbed species. This model assumes: (i) reversible adsorption (ii) no change in the properties of the adsorbed molecules (iii) no lateral interaction between adsorbed molecules (iv) one adsorption site per molecule and (v) that all adsorption sites have the same affinity for the sorbate [19]. The following represents the Langmuir isotherm equation,

$$\frac{C_e}{q_e} = \frac{1}{Q_o b} + \frac{C_e}{Q_o} \qquad \text{(Eq.5)}$$

The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal ion binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. One limitation of the Freundlich model is that the amount of adsorbed solute increases indefinitely with the concentration of solute in the solution. The empirical equation takes the form [20]

$$\log q_e = \log K + \frac{1}{n} \log C_e \qquad \text{(Eq.6)}$$

where q_e and C_e are the equilibrium adsorption capacity of the biosorbent and the equilibrium concentration in the aqueous solution, respectively. K and n are Freundlich constants related to sorption capacity and sorption intensity of adsorbents, Q_o is the maximum sorption capacity of biomass to uptake Cr(VI) (mg/g) and b is the Langmuir constant related to the energy of adsorption (l/g). The value of n falling in the range of 1–10 indicates favorable sorption. The adsorption constants and correlation coefficients obtained from the Langmuir and Freundlich isotherms are provided in (*Table 2.*).

Table 2. *Equilibrium isotherm constants of Cr(VI) biosorption by by dead B. subtilis*

Quantity of biomass used (mg)	Langmuir constants			Freundlich constants		
	Q_o (mg/g)	b (Lmg^{-1})	R^2	K	1/n	R^2
500	14.73	0.095	0.998	7.74	0.117	0.941
750	16.26	0.075	0.990	7.08	0.151	0.844
1000	16.37	0.103	0.999	7.52	0.149	0.976
1250	15.97	0.102	0.999	6.84	0.165	0.976

Cr(VI) conc. range 50-150 ppm, biomass loading range 1 - 3g/l, pH=2, Temperature=30°C.

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It is observed from (*Table 2*) that the equilibrium data are well represented by Langmuir isotherm equation when compared to Freundlich equation. The sorption equilibrium data fit Langmuir and Freundlich equation with an average R^2 value of 0.997 and 0.934, respectively. The best fit of equilibrium data for Langmuir expression confirms the monolayer coverage of Cr(VI) onto *B. subtilis* biomass

Separation factor, R_L

The essential characteristics of the Langmuir isotherms can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L, which is defined as:

$$R_L = \frac{1}{1 + bC_o} \qquad \text{(Eq.7)}$$

Where b is the Langmuir constant and C_o is the initial concentration of Cr(VI).

It is known that R_L values between 0 and 1 indicate favourable adsorption [21]. The calculated R_L values are represented in (*Table 3*).

Table 3. *R_L Values for the Adsorption of Cr(VI) onto B. subtilis biomass*

Amount of biomass (g/l)	**Initial Concentration of Cr(VI) (mg/l)**				
	50	**75**	**100**	**125**	**150**
1.00	0.17	0.12	0.10	0.08	0.07
1.50	0.21	0.15	0.12	0.10	0.08
2.00	0.16	0.11	0.09	0.07	0.06
2.50	0.16	0.12	0.09	0.07	0.06

Cr(VI) conc. range 50-150 ppm, biomass loading range 1 - 3 g/l, pH=2, Temperature=30^{o}C.

From the table, it is observed that sorption is more favorable. Also the value of R_L in the range of 0–1 at all initial chromium concentrations confirms the favorable uptake of Cr(VI) by *B. subtilis* biomass

Desorption and reuse

Desorption is a phenomenon or a process wherein some of a sorbed substance is released. The desorption of the adsorbed Cr(VI) ions from the biosorbents were studied in a batch system. The Cr(VI) ions adsorbed onto biosorbents were eluted with 0.1 M NaOH. More than 85% of the adsorbed Cr(VI) ions were desorbed from the biosorbents. In order to show the reusability of the biosorbent, adsorption-desorption experiments of Cr(VI) ions was repeated three times by using the same experimental conditions. The adsorption capacities for the biosorbent did not noticeably change during the repeated adsorption-desorption experiments. Hence this study confirms the reusable potential of the *B. subtilis* biomass.

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Mechanism of chromium removal

From the present study, it is clear that, bisorption is the mechanism of Cr(VI) removal from aqueous solution, where the anionic chromium species binds to positively charged groups of dead bacterial biomass. However, from literatures it is observed that both biosorption and bioreduction are involved in the removal of Cr(VI) from aqueous solution [22]. In order to determine the valance state of the bound chromium on the biomass, X-ray photoelectron spectroscopy was employed. The narrow scan of the $Cr^2p_{3/2}$ for Cr(VI) loaded biomass is shown in the (*Fig. 6.*).

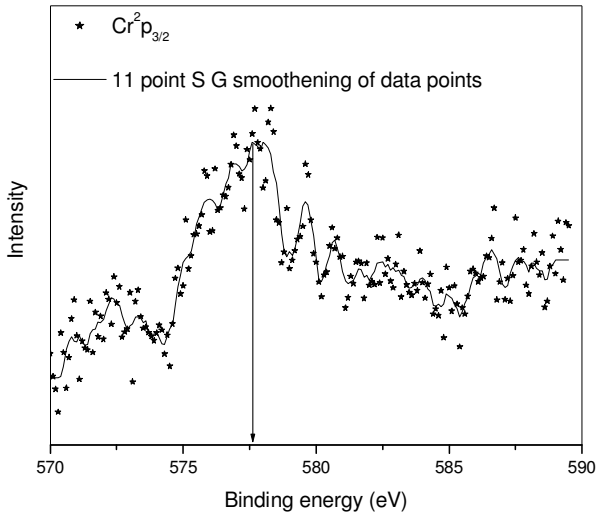

Figure 6. *$Cr^2p_{3/2}$ spectra of the chromium bound B. subtilis biomass after Cr(VI) biosorption*

There were significant amount of chromium bound on the biomass. The bands appeared at binding energies of around 577-578 eV, which corresponds to the $Cr^2P_{3/2}$ orbital of Cr(III). Based on the XPS data the existence of Cr(III) on the biomass could be inferred. The presence of Cr(VI) is also possible, however, the quantifications was not possible due to the existence of noise peaks. Park et al., [7, 23] has established two possible mechanism for the removal of Cr(VI) from aqueous solution by dead fungal biomass. According to which, the first mechanism involves direct reduction of Cr(VI) to Cr(III) in the aqueous solution by contact with the biomass. The second mechanism consists of two steps: 1) the binding of Cr(VI) to positively charged groups of the biomass and 2) the reduction of Cr(VI) to Cr(III) by adjacent functional groups having lower reduction potential value than that of Cr(VI). Thus, the present study also reports the same conclusion as the earlier studies, i.e. the most of the chromium bound on the dead bacillus biomass was in Cr(III) state.

Conclusions

Biosorption of heavy metals is one of the most promising technologies involved in the removal of toxic materials from the industrials wastewater and natural waters. The biosorption process depends significantly on the pH of the solution and is favoured at around pH value of 2.0. The biosorption process is found to be exothermic in nature. The

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maximum uptake capacity of biomass for Cr(VI) increased with the increase in initial metal ion concentration and decreased with increase in biomass concentration. Biosorption obeys the pseudo first order kinetics, which implies that the rate of biosorption process is independent of initial concentration. Intraparticle diffusion is not the rate limiting step in the biosorption process. The adsorption is well described by Langmuir isotherms that expresses that monolayer adsorption exist under the experimental conditions. The adsorption-desorption experiments were successfully carried out three times. The mechanism of Cr(VI) removal by the B. subtilis biomass was found to be adsorption-coupled reduction by employing XPS analysis. Hence, Bacillus subtilis biomass, a fermentation by-product can be used as an effective, inexpensive and alternative biosorbent for the removal of Cr(VI) from the industrial wastewaters.

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MODELING HABITAT SELECTION OF THE RED-FOOTED FALCON (*FALCO VESPERTINUS*): A POSSIBLE EXPLANATION OF RECENT CHANGES IN BREEDING RANGE WITHIN HUNGARY

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Abstract. Due to a severe population decline and shrinkage of distribution range in the past decades, the red-footed falcon has gained top priority in both worldwide and Hungarian nature conservation. As a facultative colonial breeder, in Hungary, this species predominantly nests in rookeries. The number of rooks (*Corvus frugilegus*) has also dramatically fallen recently, but population decline did not affect the large scale breeding distribution of this species. In our study we analyzed the presence of red-footed falcons at a colony in the case of current and historical breeding ranges based on landscape scaled habitat variables. We used a potential colony home-range size, estimated from observed home-range sizes in order to determine the scale of influential habitat variables. According to our results, the primary cause of the observed range shift is the urbanization of rooks in definable regions of Hungary. The ratio of forests and open water surfaces within the potential home-range had negative, while the ratio of grasslands had a positive effect on the probability of red-footed falcon presence. None of our models predicted red-footed falcon presence at colonies outside the current breeding range, suggesting that a probable increase in red-footed falcon population numbers will not be accompanied by the expansion of the current breeding range.
Keywords: *red-footed falcon, habitat, modeling, breeding range, rook, spatial GLMM*

Introduction

The analysis of habitat use and selection is important for adequate, well founded planning of species based conservation management (Pearce et al., 2008, Robles et al. 2007, Noss et al. 1997). Habitat selection and habitat use data are necessary for the prediction of a species' distribution (Elith et al., 2006, Guisan 2005, Araújo 2007), for the assessment of risk factors (Xuhezi et al. 2008, Groning et al. 2007), drafting of habitat management regulations (Garcia et al. 2006, Franco et al. 2004) and for the conservation of a single species or a group of species. Habitat selection of birds of prey is a common research topic in conservation biology (Toschik 2006, Palomino &

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Carrascal 2007, Lopez-Lopez et al. 2007, Bustamante 1997), since these birds are good environmental bioindicators (Newton 1979, Roberge & Angelstram 2004) and are often referred to as umbrella or flagship species (Sergio 2006, Ozaki 2006).

The red-footed falcon is an endangered colonial raptor species, with a continually diminishing population, classified as near threatened by the IUCN Red List (http://www.iucnredlist.org/details/144562). The breeding range is in open, typical steppe type habitats ranging from Eastern Europe to Lake Baikal in Central Asia (del Hoyo et al. 1994). This species is a long distance migrant, with presumed wintering grounds in South-Western Africa: from the Northern parts of the South African Republic through Namibia, Botswana, Angola, Zimbabwe and Zambia (del Hoyo et al., 1994, van Zyl pers. comm.).

The territory of Hungary – which is the westernmost edge of the species' distribution range (del Hoyo et al., 1994) – is almost negligible compared to its whole distribution area, but it has considerable importance in the conservation of this small bird of prey (Bagyura & Palatitz 2004). Hungarian nature conservation has always paid a great deal of attention to the protection of red-footed falcons, so the overall population estimates for the country are probably the most accurate throughout the world (Bagyura & Palatitz 2004).

The first country-wide survey performed by Keve and Szíjj (1957) in the middle of the last century estimated the breeding population to be 2200-2500 pairs, while the size of the population in 2006 was estimated at 500-600 pairs (Palatitz et al. 2006). The methods used in these surveys differed markedly, therefore direct comparison cannot be made, but it is certainly true that the population had significantly decreased – possibly by up to 50% – during the past decades (*Fig. 1.*).

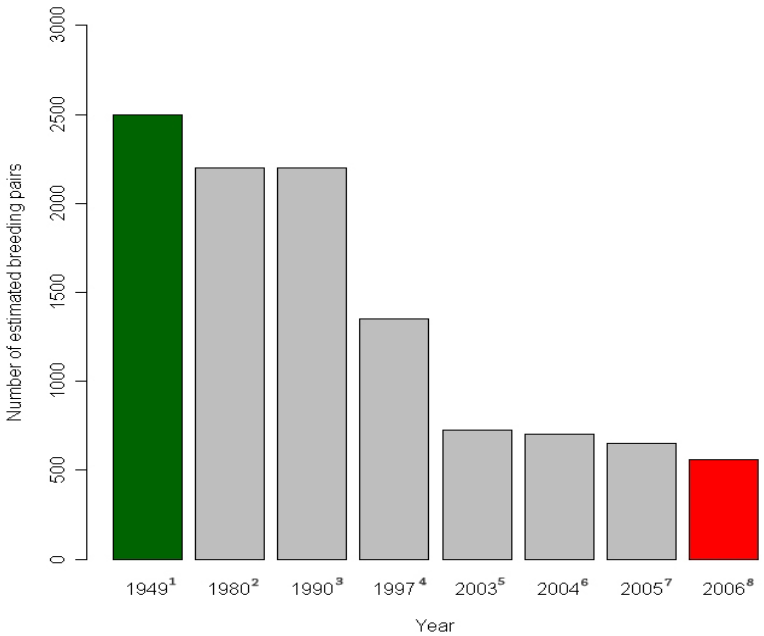

Figure 1. *The estimated maximum number of breeding red-footed falcon pairs in the past 60 years. The red and green columns reflect the number of breeding pairs of Figure2. and Figure 3., respectively.*
Source: [1]Keve & Szijj 1957, [2]Haraszthy 1981, [3]Haraszthy 1998, [4]Tóth & Marik 1999, [5,6]Bagyura & Palatitz 2004, [7]Palatitz et al., 2005, [8]Palatitz et al. 2006

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Beyond the overall population decline, the spatial distribution of breeding birds was found to be radically different during the 2006 survey (*Fig. 2.*) compared to 1949 (*Fig. 3.*). It is obvious that by 2006 red-footed falcons occupied almost exclusively the Great Plain region, practically deserting Transdanubia and Northern Hungary.

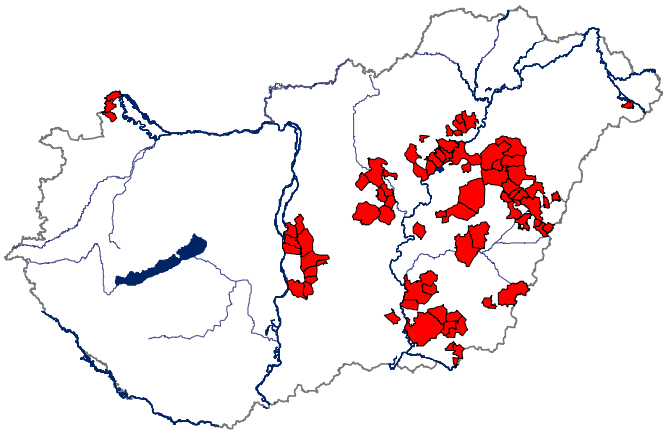

Figure 2. *The distribution of red-footed falcons in Hungary according to the 2006 survey. Red polygons mark municipipaity borders where breeding pairs were recorded in colonies or as solitary pairs.*

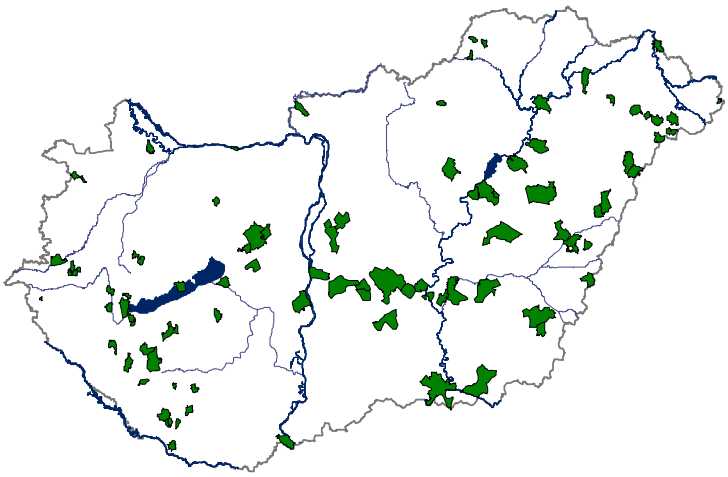

Figure 3. *Distribution of red-footed falcons in Hungary according to the 1949 survey. Green polygons mark municipality borders where red-footed falcon breeding was recorded in rookeries.*

The conditions for the colonial nesting of red-footed falcons are primarily provided by rookeries (Horváth 1964). The rook used to be a wide-spread, common species from the 1940's (Vertse 1943) to the 1980's (Kalotás & Nikodémusz 1981, Kalotás 1984), but as a consequence of an intensive eradication campaign in the mid 80's, the population rapidly decreased throughout the country (Kalotás 1987, Solt 2008) from

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about 260.000 to almost 23.000 pairs in approx. 30 years. In order to halt this trend, the rook was declared protected by the Hungarian Nature Conservation Authorities in 2001.

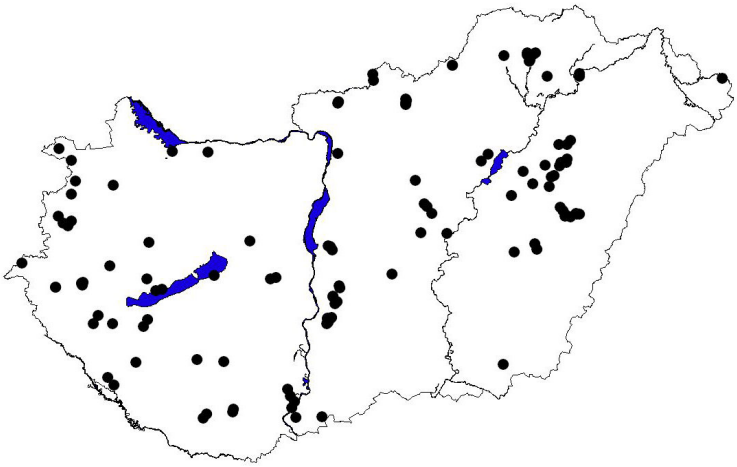

Figure 4. *Rook colonies in 2006. Despite the approx. 90% decline in the rook population, the large scale distribution of rookeries was not affected.*

As the result of the rook population decline, the number of rookeries suitable for the colonial nesting of red-footed falcons also decreased. In order to compensate for this loss, artificial nest box colonies are being established by the Life-Nature Red-footed falcon conservation project personnel (Solt et al. 2005, www.falcoproject.hu). Despite this large scale decrease in the number of breeding rook pairs, rookeries are still available for red-footed falcon nesting throughout Hungary (*Fig. 4.*). Red-footed falcon population decrease therefore can, to some degree, be explained by the crash of the rook population, but the recent changes in breeding range cannot be directly linked. In this study we analyzed the relationship between landscape scale habitat variables and the spatial distribution of colonies used by red-footed falcons in order to understand how these variables affect the current distribution pattern.

Materials and methods

As an initial step, we collected the coordinates of all colonies suitable for red-footed falcon breeding and defined the current breeding range in 2006. In the second stage we assessed the potential home-range of a red-footed falcon colony. This was carried out using habitat use analysis data deriving from a three-year radio-telemetry study. The following step was to draw the potential home-ranges around the coordinates of every colony and to intersect these with a GIS database containing habitat describing variables. The variables within the potential home-ranges of every colony were later used for statistical modelling.

Data

The geographical coordinates of colonies derived from two separate databases: the integrated population monitoring database of the red-footed falcon LIFE program, and the database of Rare and Colonial Bird species of the Hungarian Ministry of Enviorment and Water (Solt 2008).

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The analyses were carried out on two spatial scales. To be able to assess the differences between the 1949 (*Fig. 3.*) and 2006 breeding distribution (*Fig. 2.*) and to understand the pattern of colony occupation within the current breeding range, we spatially defined the "historic" and "current" breeding ranges.

We considered the whole area of Hungary as the „historic" breeding range, because the 1949 distribution (*Fig. 3.*) shows that there was at least one colony in every large region (apart from high altitude closed forests), therefore we did not a priori exclude any colonies based solely on its location. While the databases hold 198 potentially suitable colonies, we had to exclude the ones where the potential home-ranges protruded Hungary's border. Therefore 162 colonies: 41 colonies with red-footed falcon presence and 121 without red-footed falcon presence were used in the analysis

The current breeding range was defined based on the municipality borders of *Figure 2.* by applying a 500 meter buffer to the outline of the polygons and connecting the outer edges (*Fig. 4.*). Two municipality borders (on the Northwestern and Northeastern part of the country) were excluded from the current breeding range because they hold only four solitary pairs altogether. Therefore, the defined current breeding range holds all colonies occupied by red-footed falcons and over 99% of the solitary pairs.

Defining the potential home-range of a colony

The true home-ranges of individual red-footed falcons were estimated from the data of the LIFE program's ongoing habitat preference analysis. We radio-tagged 24 birds in the 2006 and 2007 breeding seasons with 3.5g "Biotrack TW-4" radio-tags. The birds were directly followed during their hunting, and the exact location of hunting events and other habitat use variables were recorded (see methods in: Franco et al. 2007, Tella et al.1998).

Based on the individual home-ranges, we estimated the potential home-range of a colony to be a 3000 meter radius circle, which covers at least 95 % of the localization points of the studied colonies (unpublished data).

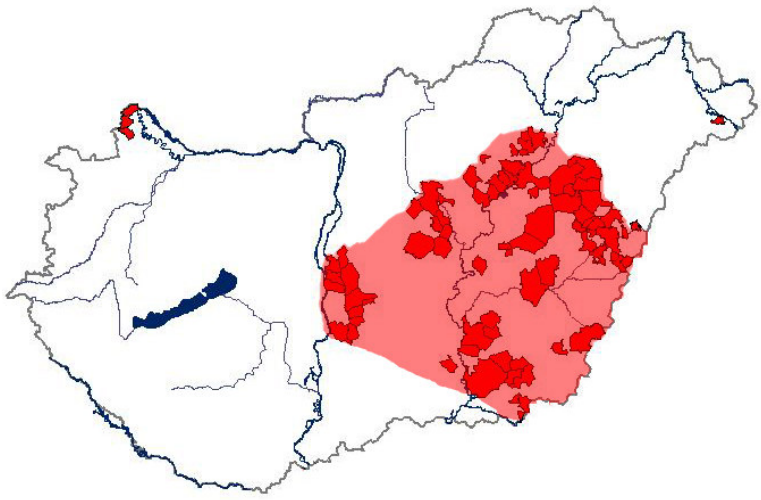

Figure 5. *Defining the „current" breeding range of red-footed falcons. The red polygons shown on the map mark the municipality borders where red-footed falcon breeding occurred in 2006 according to the monitoring data. The ruby red large polygon shows the area defined as the „current" breeding range in our analyses.*

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Habitat variables

The habitat variables were extracted from the CORINE land cover frameworks GIS database. In Hungary, this database was created by SPOT4 Xi+M type satellite images shot in 1998 and 1999. The CORINE has 79 land cover categories. The minimum area of uniform polygons is 4 ha. Although the database is relatively out of date, it is still usable to analyse certain variables due to its coarse resolution. The 79 variables were summed and transformed into 12 biologically relevant variables (*Table 3.*).

The potential home-range size (3000 meter radius circles) was used to obtain the spatial explanatory variables surrounding each colony. We used the size, length and number of the habitat categories within the potential home-ranges for the analyses.

Table 1. *Spatial variables used to model landscape scale breeding colony occupancy of red-footed falcons*

Variable	Unit
Water canals	Meter
Forest	Ha
River	Ha
Road	meter
Grassland	Ha
Small parcel arable land	Ha
Large parcel arable land	Ha
Farms	pcs
Settlements	Ha
Railroad	meter
Water surface	Ha
Wetlands	Ha

Statitstics

We used Spatial Generalized Linear Mixed Effects Models (Spatial GLMM) with logit link and binomial response to analyse the presence/absence of red-footed falcons in relation to landscape scaled habitat variables on both historic and current breeding range scales. Model selection was carried out by applying a decision tree (CART model) to all variables. The variables grouping the observations were used as explanatory variables in the Spatial GLMMs. Decision tree pruning was carried out by optimizing the complexity parameter (Faraway 2005). We categorized the variables into 5 ascending categories to aid better understanding of decision tree outputs, while we used the relative size of the same variables in the GLMMs. We fitted a spatial GLMM on the observations when the explanatory power of the decision tree was sufficient and the hierachical structure of the grouping variables was biologically interpretable. The explanatory power of the GLMMs was more or less smaller than that of the decision trees, possibly due to the spatial autocorrelation of the expalanatory variables. The CART models were unable to differentiate between artificial and natural colonies (decision tree, Cohen's $\kappa = 0.23$, 95% Confidence Interval:-0.02, 0.47), therefore we did not distinguish colony types in the analyses.

We used the QGIS sofware (www.qgis.org) to handle and map GIS variables, and the R 2.8 sofware for data analysis (R Development Core Team 2007). The most important R packages and their role in the analysis are presented in *Table 2*.

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Table 2. *Most important R packages and their role used in the analyses*

Package	Role	Authors
Adehabitat	Home range estimation	Calange
aspace	Measuring spatial distance	Remmel & Buliung
spdep	Measuring spatial autocorrelation	Bivand
mvpart	CART models	Therneau & Atkinson
MASS	Spatial GLMM	Venables & Ripley

Results

„Current" breeding range

The grouping variables of the decision tree applied on the current breeding range scale were: „Forest", "Large parcel arable lands" and "Grasslands". The spatial GLMM fitted with these variables correctly classified 74 % of the observations (Cohen's $\kappa = 0.48$, 95% Confidence Interval: 0.3, 0.67). The "Large parcel arable land" variable had no siginifcant effect, the "Grassland" variable had marginally significant positive effect, while the "Forest" variable had significantly negative effect, according to the model (*Table 3.*).

Table 3. *The output of the spatial GLMM fitted on the presence/absence of red-footed falcons at a colony within the „current" breeding range.*

	estimate	SE	t-value	p-value
Intercept	-1.37	1.53	-0.89	0.3746
Grassland	0.03	0.019	1.68	0.0599
Forest	-0.52	0.20	-2.58	**0.0164**
Large parcel arable lands	0.013	0.019	1.01	0.4951

Although the „Large parcel arable land" had a significant grouping effect in the decision tree, it turned out to be non-significant in the spatial GLMM. This is probably due to the strong spatial autocorrelation of this variable. Presumably, decision trees overestimate the grouping effects of highly autocorrelated variables, hence the contradiction of the role of this variable in the different statistical analyses.

„Historic" breeding range.

The decision tree applied on the „historic" breeding range scale (i.e. the whole country) classified 90% of the observations correctly. The grouping variables were: „Roads", „Water surface", „Forest" and „Grassland". The spatial GLMM fitted with these variables classified 87% of observations correctly (*Table 6.*). The classification of this model is also significantly deviating from random classification (Cohen's $\kappa = 0.52$, 95% Confidence Interval: 0.51, 0.78). All grouping variables of the decision tree stayed significant in the spatial GLMM.

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Table 4. *The output of the spatial GLMM fitted on the presence/absence of red-footed falcons at a colony within the „historic" breeding range.*

	estimate	SE	t-value	p-value
Intercept	-3.33	1.16	-2.87	0.005
Forest	-1.5	0.33	-4.52	<0.001
Grassland	0.042	0.01	3.53	<0.001
Road	-0.19	0.085	2.21	0.023
Water surface	-1.07	0.4	-2.63	0.008

The significant „Road" variable is highly correlated with the ratio of „Settlement" variable (Spearman's rank correlation coefficient = 0.75, $p<0.0001$) within the potential home range, therefore it can be used as an indicator of human presence. If the „Settlement" variable occupied more than 25% percent of the potential home-range of a given colony, we classified it as urban colonies. Out of the 76 rookeries outside the current breeding range, 58 are classified as urban colonies. On the other hand, only 3 out of the 86 colonies within the current breeding range are classified as urban colonies. The „Forest" variable had a negative effect on the probability of red-footed falcon presence at a given colony on both spatial scales, although there is a large difference in the ratio of forests between the two breeding ranges. The „historic" model does not predict red-footed falcon presence at any of the colonies outside the current breeding range (*Fig. 6.*).

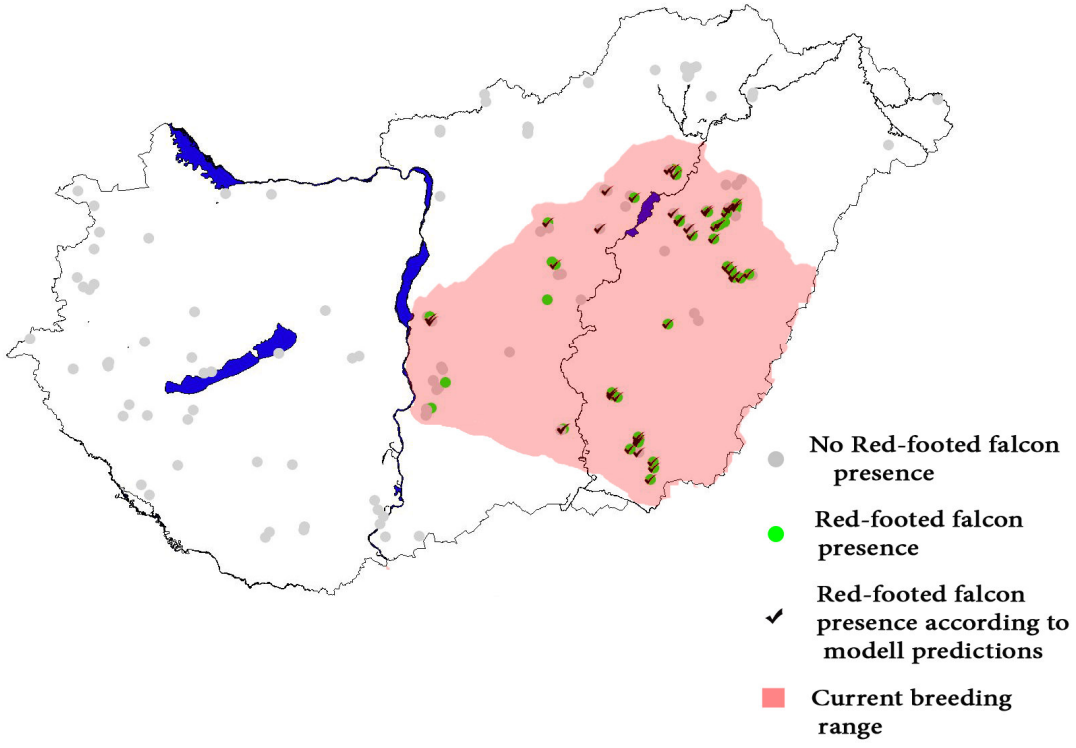

Figure 6*. The predictons of the historic breeding range model. According to our model predictions, there are no colonies suitable for red-footed falcon breeding outside the current breeding range.*

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Discussion

Although the classification of the model fitted on the current breeding range is moderately accurate (74%), it reveals an interesting pattern in the case of the "Forest" variable. This variable has significant negative effect on the probability of red-footed falcon presence in models fitted on both spatial scales, even though the variable range is quite different. The CORINE can only differentiate forest patches larger than 4 ha, therefore the 3-4% of forests in the potential home ranges of colonies within the current breeding range practically mean a few small patches or one larger patch. The Goshawk (Accipiter gentilis) is a regular, well distributed (Haraszthy 1988) predator of the red-footed falcons (pers. obs., Bagyura & Haraszthy 1994), occupying small to large forest patches for breeding. Therefore, the observed colony occupying pattern of red-footed falcons can be explained as a predator avoiding strategy (e.g. Brodie & Formanowicz 1991, Fontaine & Martin 2006).

The positive effect of grasslands was not as significant as expected in the current breeding range model. The habitat use analyses revealed that this species uses agricultural lands relatively often for hunting (Fehérvári et. al 2006), suggesting that the birds can substitute grasslands to a certain degree, similar to lesser kestrels (Tella et. al 1998), causing the "Grassland" variable's lower explanatory power. The significant difference between colonies outside the current breeding range and the ones currently occupied by red-footed falcons can be explained by two reasons: 1) the previously mentioned urbanization of rooks outside the current breeding range, and 2) that the landscape has been significantly transformed over the past decades. Source: Central Statistical Institute: http://portal.ksh.hu/pls/ksh/docs/hun/agrar/html/tabl1_3_1.html). Presumably, landscape modification has been greater outside the current breeding range, but most probably these two main causes acted synergistically to generate the current red-footed falcon breeding distribution. This presumption may be confirmed by the prediction map of the "historic" breeding range model, which does not predict red-footed falcon occurrence outside the current breeding range (*Fig. 5.*). Although our model variables derive from a coarse GIS database, and we only considered the distribution pattern of one year's red-footed falcon breeding distribution, our model predictions may aid the mid-term nature conservation strategy of this near threatened species. It is quite clear that – in the current situation – without the local redistribution of rookeries (i.e. from urban to natural habitats outside the current breeding range of red-footed falcons) there is a low chance of red-footed falcon breeding range re-expansion.

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SIMULATIONS OF THEORETICAL ECOSYSTEM GROWTH MODEL (TEGM) DURING VARIOUS CLIMATE CONDITIONS

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Abstract. Climate change has a great impact on the build and the work of natural ecosystems. Disappearance of some population or growth of the number in some species can be already caused by little change in temperature. A Theoretical Ecosystem Growth Model was investigated in order to examine the effects of various climate patterns on the ecological equilibrium. The answers of the ecosystems which are given to the climate change could be described by means of global climate modelling and dynamic vegetation models. The examination of the operation of the ecosystems is only possible in huge centres on supercomputers because of the number and the complexity of the calculation. The number of the calculation could be decreased to the level of a PC by considering the temperature and the reproduction during the modelling of a theoretical ecosystem and several important theoretical questions could be answered.
Keywords: *climate modelling, ecosystem, climate change, theoretical ecological model*

Introduction

The important community-ecological researches have three main approaches related to methodology considering the climate change. The ecologists working in the fields observing the real natural processes have aspired to take the fewest possible interferences to their processes (Spellerberg, 1991). The aim is to describe the community ecological patterns unbiased (Juhász-Nagy P.).

The other school of ecological researches examines hypothesises about the natural processes. The basis of these researches is to test the differential prediction in manipulative trials (Précsényi, I, 1995). The third part of ecologists deals with modelling where a precise mathematical model is made for basic and simple rules of the examined phenomena.

The work of the modelling ecologists consists of two parts. The first is to test the mathematical model with case studies and the second is to develop (repair and fit again) the model. Nowadays these available models are far away from the observations of field ecologists.

It is obvious that all the three approximations have advantages and disadvantages. There are two approaches: monitoring and hypothetic central approximations. In the course of monitoring approaches the main purpose is to discover the relationships and patterns among empirical data. This is a multi dimensional problem where the tools of the biomathematics and statistics are necessary. The data are originated from large

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monitoring systems (e.g. national light trap network, Long Term Ecological Research (LTER)).

There are hypothetical central facts where the known or assumed relationships mean the starting point. There are three types of the researches in this case:

- Testing simple hypotheses with laboratory or field experiments (e.g. fitotron plant growing room)
- Analyzing given ecosystems with tactical models (e.g. local case studies, vegetation models, food net models, models of biogeochemical cycles) (Fischlin et al., 2007; Sipkay et al., 2008; Vadadi-Fülöp et al., 2008)
- Examination of general questions with strategic modelling (e.g. competition and prediction models, cell-automats, evolutionary-ecological models)

Our aim is to analyze the effect of some temperature climate-patterns to the production and common-ecological relations in a strongly simplified theoretical model.

There is an important task is to save and protect the biodiversity beside climate change. It is generally can be said that the living beings do not react uniformly on the change in their environment. During the history of the Earth the climate changes have great impact on the composition of the flora and the fauna. The decrease in biodiversity is caused by a decrease in the number of species.

There is an important task is to research the relationship between the biodiversity and climate change. Our aim is to analyze the effect of some climate-patterns to the production and diversity with a strongly simplified theoretical model (TEGM).

Material and methods

Theoretical Ecosystem Growth Model (TEGM)

During our examinations the behaviour of a theoretical ecosystem is studied on various changing of the temperature. The simulation was made by Excel with simple mathematical background. An algae community in a terrestrial freshwater ecosystem is modelled by the theoretical ecosystem.

The algae species are characterized by the temperature interval in which the given species are able to reproduce. This reproductive feature depends on their temperature sensitivity. There are four types of species related to their sensitivity: super-generalists (SG), generalists (G), transitional species (T) and specialists (S). The temperature-optimum curve is originated from the normal- (Gaussian-) distribution, where the expected value is the temperature optimum. The dispersion depends on the niche-overlap among the species. The overlapping is set on a way where the results agree with the niche overlap of the lizard species studied by Pianka (1974) where the average of the total niche overlap decreases with the number of the lizard species.

$$Niche\ overlap=(niche\ separation/\ niche\ spread)=(\mu_1-\mu_2)/\sigma \qquad \text{(Eq.1)}$$

The examined temperature range agrees with the temperature variation in the temperate zone. 33 algae species with various temperature-sensitivity can be seen in the 1st figure. The daily reproductive rate of the species can be seen on the vertical axis which means with how many times the number of specimens can increase on a given temperature. This corresponds to the reproductive ability of freshwater algae in temperate zone (Felföldi, 1981; Reynolds, 2006; Sipkay et al., 2007).

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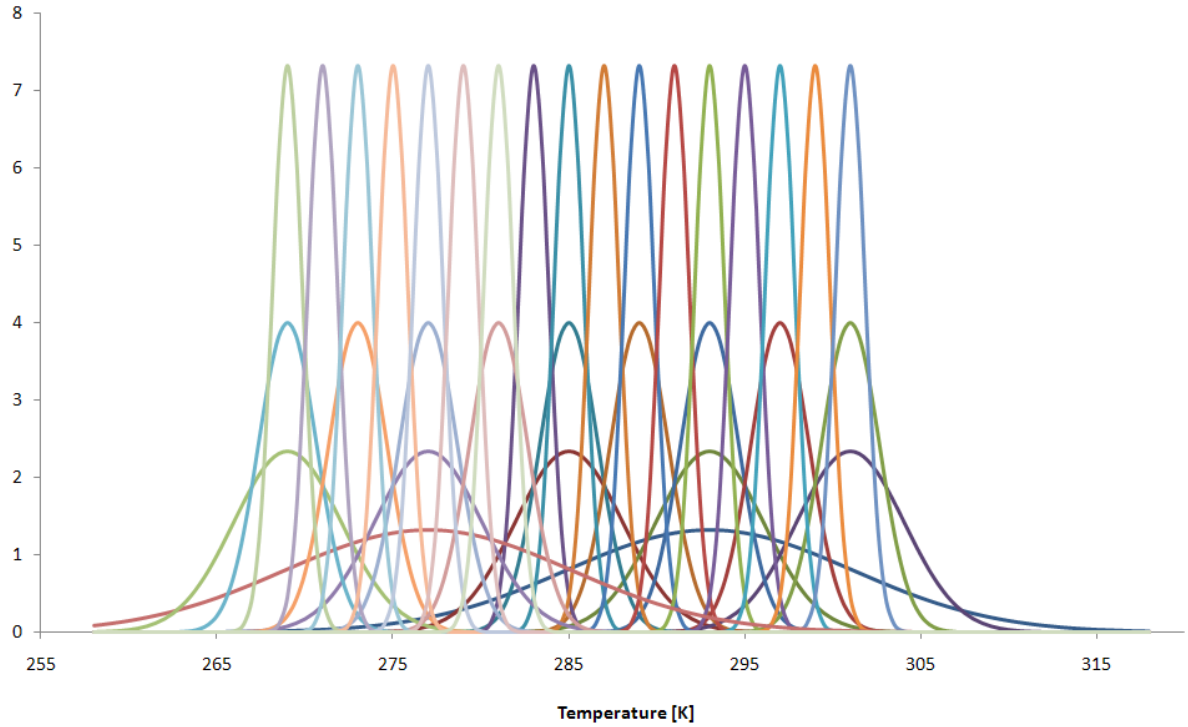

Figure 1. *Reproductive temperature pattern of 33 algae species*

The 33 species are described by the Gaussian distribution with the following parameters:

- 2 super-generalists (μ_{SG1}=277 K; μ_{SG2}=293 K; σ_{SG}=8.1)
- 5 generalists (μ_{G1}=269 K; μ_{G2}=277 K; μ_{G3}=285 K; μ_{G4}=293 K; μ_{G5}=301 K; σ_{G}=3.1)
- 9 transitional species (μ_{T1}=269 K; μ_{T2}=273 K; μ_{T3}=277 K; μ_{T4}=281 K; μ_{T5}=285 K; μ_{T6}=289 K; μ_{T7}=293 K; μ_{T8}=297 K; μ_{T9}=301K; σ_{T}=1.66)
- 17 specialists (μ_{S1}=269 K; μ_{S2}=271 K; μ_{S3}=273 K; μ_{S4}=275 K; μ_{S5}=277 K; μ_{S6}=279 K; μ_{S7}=281 K; μ_{S8}=283 K; μ_{S9}=285 K; μ_{S10}=287 K; μ_{S11}=289 K; μ_{S12}=291 K; μ_{S13}=293 K; μ_{S14}=295 K; μ_{S15}=297 K; μ_{S16}=299 K; μ_{S17}=301 K; σ_{S}=0.85).

Since the reproductive ability is given the daily number of specimens related to the daily average temperature is definitely determinable. We suppose 0.01 number of specimens for every species as starting value and the following minimum function describes the change in the number of specimens:

$N(X_i)_1 = 0.01$ for every i=1,2, …, 33 species,

where $N(X_i)$: the number of specimens of the i^{th} species

$$N(X_i)_j = N(X_i)_{j-1} \cdot Min\left\{ RR(X_i)_j ; a\left(1-\left(\frac{\sum_{i=1}^{33} N(X_i)}{K_j}\right)^{v}\right)^{r} \right\} + 0{,}01, \qquad \text{(Eq.2)}$$

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where

j :is the number of the days (normally j=1,2,…,3655)

$RR(X_i)_j$: is the reproductive rate of the X_i species on the j^{th} day

a=8

ν=0.8

r: is the velocity parameter (r=1 or 0.1)

$K_j = d_1 \cdot \sin(d_2 \cdot k + d_3) + d_4 + 100000 \cdot Rnd() - 50000$, this is the restrictive function of reaching the sunlight.

k=1,2,…,366 , this is the sequential number of the given year (year-day)

d_1=4950000, d_2=0,0172, d_3=1,4045, d_4=5049998

The constant values of the K_j restrictive function is set in a way where the period of the function is 365.25, the maximum place is on 23rd June and the minimum place is on 22nd December.(These are the most and the least sunny days.)

In our researches the distribution of the algae community of a theoretical freshwater ecosystem is examined under changing the temperature. Rivalry begins among the species with the change of temperature. In every temperature there are dominant species who win the competition. The temperature was changed according to plan in order to estimate the various effects separately. The duration of the simulation was usually 10 year in the experiments. Two experiment series were run related to the dual value of the velocity parameter.

The effect of the existing climate patterns (historical or future daily temperatures) was analyzed.. The daily average temperature data are periodic through years; therefore appropriate functions were made to describe the climate.

The output parameters of the experiments are annual complete number of species and the related Shannon diversity index.

Results

Historical data (Budapest, 1960-1990)

The equilibrium has been reached; the competition of the species can be seen in *Fig.2-3*. The faster ecosystem has a lot of specialists and some transient species. In summer there is stationer temperature therefore the slower and the faster ecosystems show quite similar picture (S13, T7, G4 and SG1 species), winter the K2 (light blue) species occurs.

Future (Budapest, 2070-2100)

According to the A2 scenario (HC adhfa) there is no optimum reproductive temperature for any species during the slower or the faster (*Fig. 4.*) processes. In winter there will be more species such as G2, T3, S5 and S6.

According to the B2 scenario (HC adhfd) the G5 species has optimum reproductive temperature in summer.

Comparing the Max Planck Institute (MPI) and the Hadley Centre (HC) predictions for 2100 it is stated that the seasons do not separate from each other related to the contents of the ecosystem.

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The Shannon diversity values for the various climate patterns can be seen in *Fig. 5.* In the figure the number of all specimens are shown for the slower and the faster ecosystem.

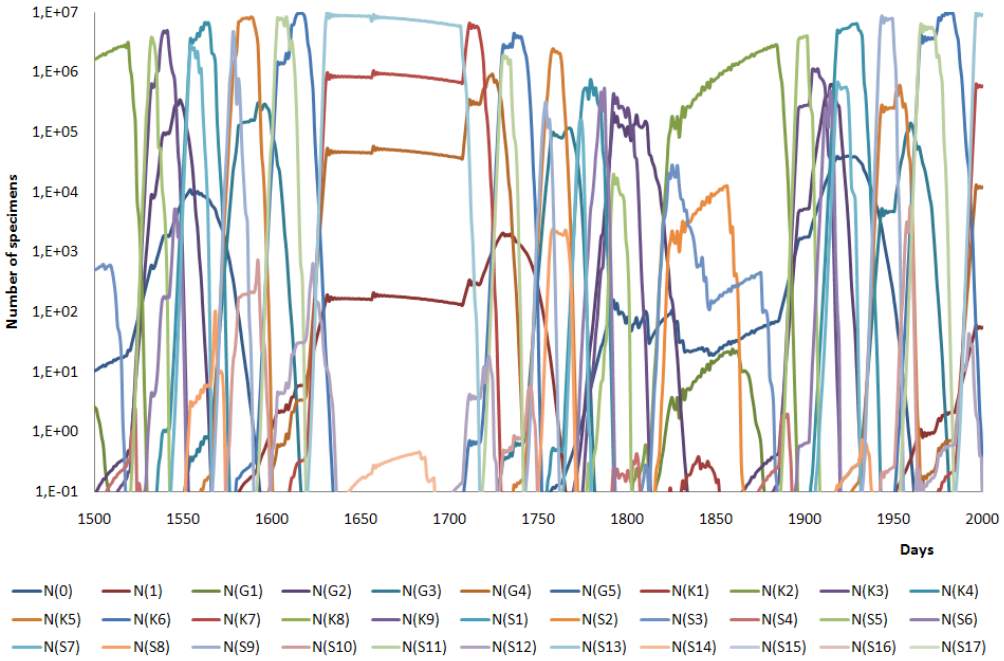

Figure 2. *Appereance of all species under historical climate of Budapest (1960-1990) between the 1500th and the 2000th day of simulation (velocity factor is equal to 1)*

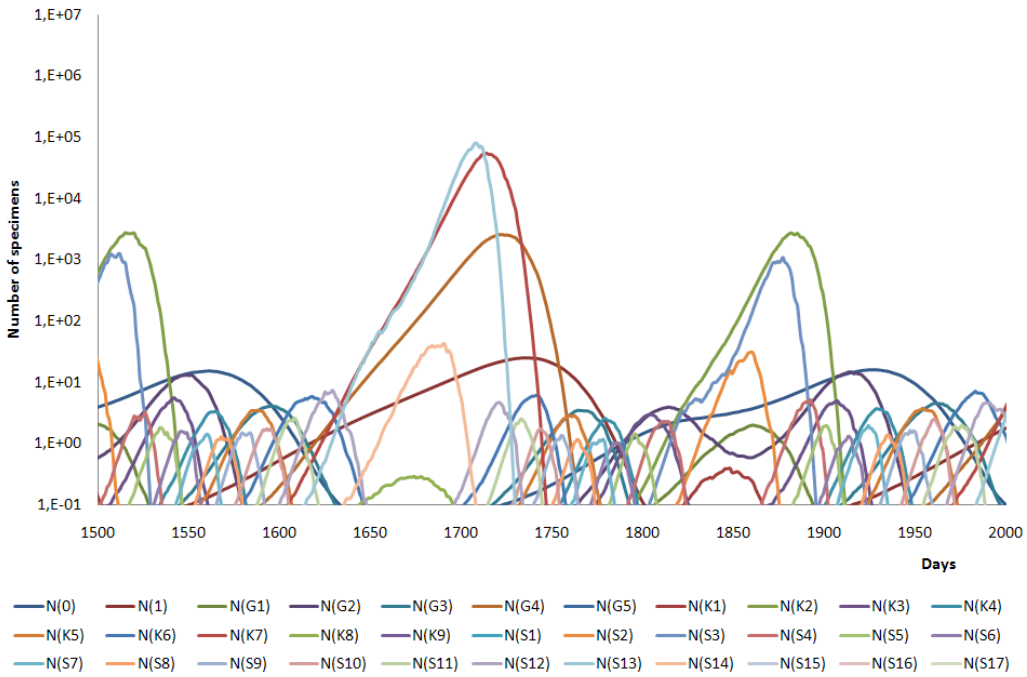

Figure 3. *Appereance of all species under historical climate of Budapest (1960-1990) between the 1500th and the 2000th day of simulation (velocity factor is equal to 0.1)*

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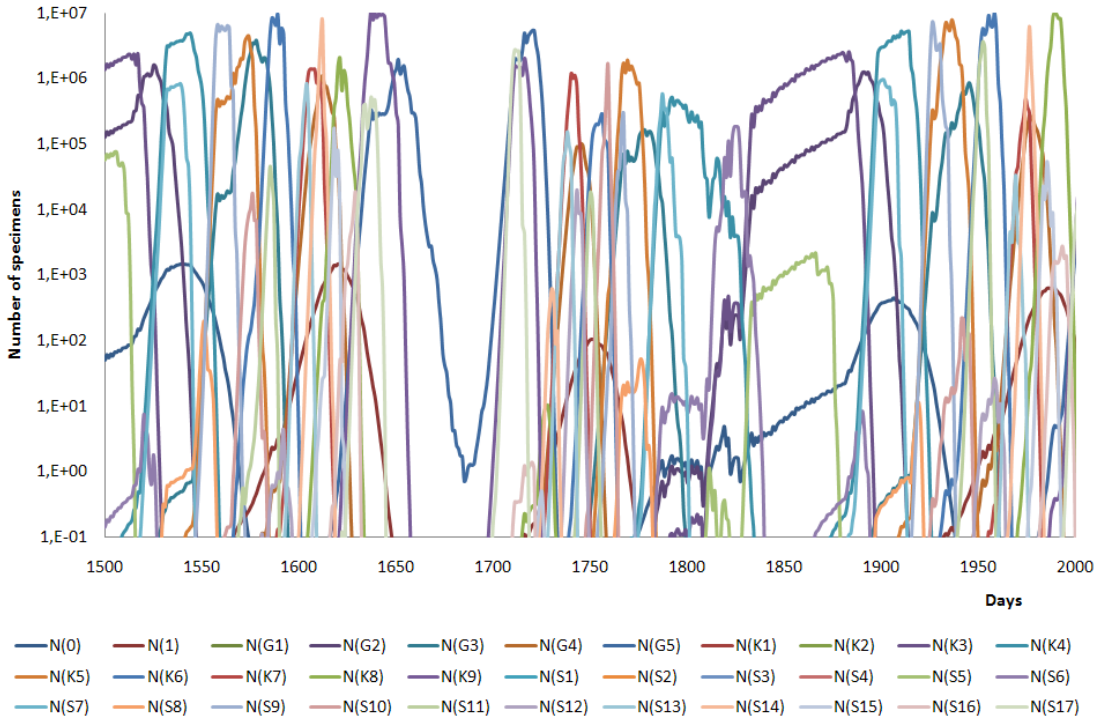

Figure 4. *Appereance of all species under future climate of Budapest (HC adhfa, 2070-2100) between the 1500th and the 2000th day of simulation (velocity factor is equal to 1)*

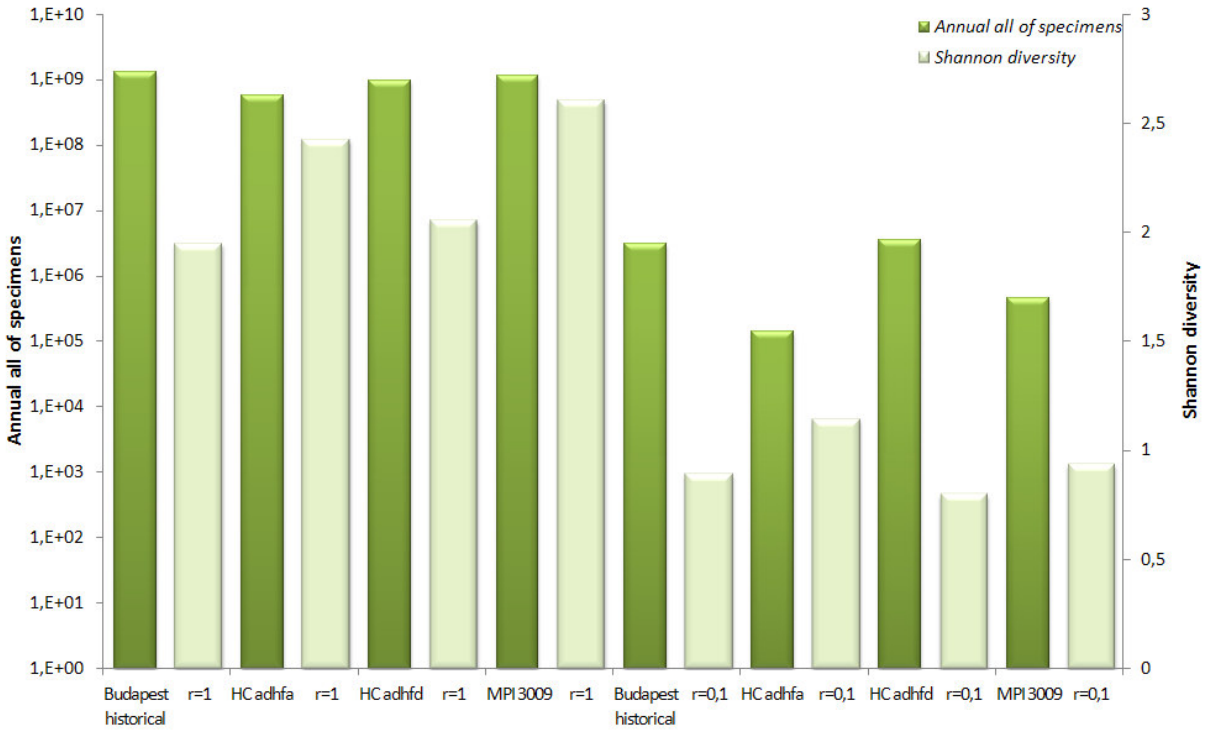

Figure 5. *Annual complete number of specimens and diversity in the course of various climate patterns*

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Conclusion

The number of all specimens in a year decreases for a given species in the future (estimated weather data series by 2070-2100, MPI 3009, HC adhfa and HC adhfd), the amount of decrease is different among the various results.

Regarding diversity the annual value of the Shannon index increases towards the future (in case of HC adhfa and MPI 3009 data series), but the HC adhfd prognosis shows the same pattern with the historical data (Budapest, 1960-1990). These processes are similar during the slower and the faster ecosystem. The diversity value of the slower process is the half of the faster's.

Summary

In the course of our simulations has been shown what kind of effects are there in the composition and competition of an ecosystem with the change of temperature. The specialists reproducing in narrow temperature interval are dominant species in case of constant or slowly changing temperature pattern but these species disappear under small fluctuation in the temperature.

Comparing the Hungarian historical data with the regional predictions of huge climate centres (HC, MPI) it is stated that the newer estimations (such as HC adhfa, HC adhfd and MPI 3009) show a decrease in number of specimens in our theoretical ecosystem.

The ecosystems make an important role in the biosphere in development and maintenance of the equilibrium. Regarding the temperature patterns not only the climate environment affects the composition of ecosystems but the plants make a feedback to their milieu throughout the photosynthesis and respiration in the global carbon cycle.

The specimens of the ecosystems do not only suffer the change in climate but they could affect the equilibrium of the biosphere or the composition of the air through the biogeochemical cycles. There is an opportunity to examine the controlling ability of temperature-climate with the theoretical ecosystem.

In our further research we would like to examine the effect of the ecosystem back to the climate. These temperature feedbacks have got a great emphasis related to DGVM models with large computation skills (Friedlingstein et al., 2006), but the feedbacks are not estimated directly. We would like to examine the process of the feedback with PC calculations to answer easy questions.

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APPLICATION OF ORIBATID MITES AS INDICATORS (REVIEW)

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Abstract. This review discusses the connection between quantitative changes of environmental factors and oribatid communities. With the overview of available studies, it can be clearly explored how various characteristics of Oribatid communities are modified due to changes in moisture, temperature, heavy metal concentration, organic matter content and level of disturbance. The most important question concerning the application of Oribatids as indicators is to clarify what kind of information content does natural Oribatid coenological patterns possess from the aspect of bioindication. Most of the variables listed above can be directly measured, since rapid methods are available to quantify parameters of the soil. Responses of Oribatids are worth to study in a more complex approach. Even now we have an expansive knowledge on how communities change due to modifications of different factors. These pieces of information necessitate the elaboration of such methods which render Oribatid communities suitable for the task to prognosticate what extent the given site can be considered near-natural or degraded, based on the Oribatid composition of a single sample taken from the given area. Answering this problem needs extensive and coordinated work.
Keywords: *community, bioindication, soil, ecology, coenology*

Introduction

Applicability of Oribatid mites as an indicator group has been emphasized by researchers for several decades. These organisms possess such kind of extraordinary characteristics by which (considered even separately or as a whole) they are able to indicate different changes in their environment. These characteristics have been summarized in several reviews, most thoroughly in the works of Lebrun and van Straalen (1995), Behan-Pelletier (1999) and Gulvik (2007).

Oribatid mites can be found in almost every kind of habitats worldwide: on land, water and most importantly in the layers of soil containing organic materials, but they also conquered several other kind of microhabitats (e. g. lichen, moss, treebark etc.). Apart from the diversity of habitats, their excessive adaptational ability is also shown by great abundance and species richness. In most habitats, they constitute the largest proportion of microarthropods. These characteristics mentioned above can be primarily used in the application of coenological methods.

Oribatid mites consume mainly living or dead parts of plants or fungi, however there are some predators and scavengers to be mentioned as exceptions (Behan-Pelletier, 1999). As a consequence, they consume variuos kinds of food, and as such, they participate in numerous ways in the structure of the food web (Lebrun and van Straalen, 1995). Thus they are in strong interaction with their microenvironment (e. g. Ca-storage, heavy metal accumulation (Norton and Behan-Pelletier, 1991, Behan-Pelletier

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1999), play an important role in the forming of soil structure and decomposition processes (Behan-Pelletier, 1999). These features can be applied for indicating the effects of chemical or heavy metal pollutions, and disturbances in the succession of decomposition processes (Lebrun and van Straalen, 1995).

The reproduction biology and life cycle of Oribatid mites can be considered extraordinary among arthropods from several aspects. There are some species/populations with sexual and asexual reproduction, and the proportion of species with obligate thelytokous parthenogenesis is very high – around 10% (Lebrun and van Straalen, 1995). Iteroparity and multiannual life cycle are also quite prevalent among the species, especially in moderate and cold climate zones (Norton, 1994, Luxton, 1981, Behan-Pelletier, 1999). The slow development, low fecundity and long larval stage of Oribatid mites can help indicating long-term disturbances. Their low dispersion ability (Lebrun and van Straalen, 1995) is also quite important, since these mites can hardly flee from sites affected by some kind of stress. Oribatid mites are classified as a „K-selected" group; this can be lead back to their slow metabolism according to Norton (1994).

Based on the characteristics listed above, many researchers think that this group is quite promising since it can be used for various indication purposes. However, indication purposes can be quite different; two main types can be formed on the basis of avilable studies:

The first type examines the communal or individual characteristics of Oribatid mites as a function of a well-defined variable. Such variables are water content, temperature, heavy metal concentration and organic matter amount, these factors are included in this study. The second type compares Oribatid communities via habitats distinguished by complex factors and features. Studies of consequences of human activities or comparisons of different habitats belong to this type.

The following section discusses the connection between quantitative changes of environmental factors and oribatid communities, then the results of complex comparisons are summarized.

Change in a simple factor

Soil moisture (water content)

Soil moisture is one of the most decisive factors affecting the life of Oribatid communities. Two types of studies examining this factor exist. One of them monitors the change in seasonal precipitation as a field description via soil moisture, the second type applies irrigation experiments. Depending on the circumstances of the studies, different results has been obtained, though almost all of the studies support the theory that Oribatid mites generally like habitats with elevated humidity and they are susceptible to drought.

It can be observed in tropical areas, both in the primary (Trueba et al., 1999) and secondary (Badejo and Akinwole, 2006) rainforests that the density of Oridatid mites in soil samples has been much greater in the rainy season than that in the dry season. In the study of Noti et al., (2003) water content has been a key factor affecting the species richness of Oribatid mites, however its effect varied between seasons. Beside soil moisture, C-content, C/N ratio and N-content are also important factors. Higher soil moisture results in greater density (Noti et al., 2003, Lindo and Winchester, 2006, Melamud et al. 2007). Lindo and Winchester (2006) showed that spreading of species

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on the soil and in the foliage of trees is limited primarily not by physical barriers but e. g. water content. It has to be mentioned however, that the study of Melamud et al. (2007) showed increasing species richness proceeding upwards on Mt Carmel (Israel), while the moisture gradient has grown downwards.

Tsiafouli et al. (2005) conducted short-term manipulation studies in Mediterranean sites. Various irrigation and drying methods have been applied and it was shown that drought decreased the species richness of Oribatid and Collembolan communities (differences in abundance were not significant), while irrigation increased diversity of both groups. This phenomenon could have been caused by the propagation of rare species after irrigation (Tsiafouli et al., 2005).

In another study however, irrigation lowered the abundance of mesofaunistic units (O'Lear and Blair, 1999), but this study has been conducted on prairie and has not focused on Oribatid mites. This study also pointed out that if a soil sample from a humid site was placed into the soil in an arid area, only the abundance of the Oribatid group decreased, which can be considered as a further proof of this group's drought susceptibility.

Lindberg et al. (2002) conducted extensive studies in Swedish coniferous forests concerning drought effects. He also pointed out that long-term deprivation of precipitation decreases the abundance of Oribatid mites and the diversity of the community (Lindberg et al., 2002). Lindberg also examined what kind of long-lasting effects have been caused by draught to the community and how long the regeneration would take: he could not measure similar results even three years after the intervention comparing treated and untreated control sites. Besides, he pointed out that Oribatid mites are more sensitive and possess much moderate regeneration ability compared to Collembola or Mesostigmata (Lindberg and Bengtsson, 2006). Lindberg et al. (2002 and 2006) listed several supposed causes of high susceptibility: in some lifestages Oribatid mites are very sensitive to drought, larger species can not wander deeper into the soil, so they have an inferior dispersion ability. Additionally, biomass and diversity of fungi also decrease as an effect of draught and fungi present a food source for many Oribatid species. Moreover, oviposition of some species are connected to special fungi (Hågvar, 1998, Lindberg et al., 2002). Lindberg and Bengtsson (2005) described that there are differences among Oribatid groups in drought tolerance. Species with sexual reproduction and species with less prevalence have a better drought tolerance than generalists and parthenogenetics.

It has been found that additional water supplement had not affected Oribatid communities when desert species had been investigated (Cepeda-Pizarro et al., 1996). According to studies conducted in the Namib desert, C/N ratio and K-content had greater effect than water content. Taylor and Wolters (2005) observed difference between the drought tolerance of species living in the litter of coniferous and beech forests. Oribatids in coniferous forests were more susceptible to drought than species living in the litter of beech forests. Source limitation and deterioration of nutrient availability commence sooner in coniferous forest litter. According to Taylor and Wolters (2005), drought can be shown via the decrease in abundance values, but many of the studies mentioned above state that diversity also falls as a consequence of decreasing species richness.

In accordance with the aforementioned, we summarize the results of some observations regarding which part of the Oribatid group can be characterized with drought susceptibility, and what are the causes of this susceptibility. Only a few studies

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mention species with proven drought tolerance or susceptibility. Papers describe Zygoribatula exilis, several Entomobrya spp. (Lindberg et al., 2002), Chamobates borealis and Tectocepheus velatus as heavily affected by drought, while Disshorina ornata is characterized as drought tolerant (Taylor and Wolters, 2005). Species with sexual reproduction and narrow habitat preference are more tolerant to drought (Lindberg and Bengtsson, 2005) compared to species with greater size and living in the soil surface or forest litter (Lindberg et al., 2002) and those Oribatids which slowly colonized the offered litter in a litter colonization experiment (Taylor and Wolters 2005). The work of (Walter and Proctor, 1999, Taylor et al., 2002, Taylor and Wolters, 2005) states that adult individuals are able to tolerate a wide range of water content, but nymphs are quite susceptible to drought (Taylor and Wolters, 2005). According to the same study, water content does not directly affect Oribatids, drought exerts it effect via fungi and microbes which serve as food for the Oribatids. Thus, drought susceptibility exerts its effects via food limitation and Oribatids can indicate water deficiency in an indirect manner, affected by the decrease of the quantity of microbiota.

Temperature

Most of the studies examining the effects of temperature are manipulation experiments including both field and laboratory studies. The aim of these studies had been mostly to observe the effects of temperature change or fluctuation accompanying climate change on mite species and communities. These experiments dealt with polar habitats and coniferous forests.

A part of the experiments studied the frost tolerance of mites, and the effects of temperature fluctuation near the frost limit. Sulkava and Huhta (2003) started from the presumption that global warming shortens the period of snow cover, harmfully affecting animals in the soil by exposing them to greater fluctuation of temperature and aggravating the danger of frost. In field studies, snow cover in the coniferous forest was artificially removed, and consequently the species richness of microarthropoda decreased. In laboratories, the sample populations were exposed to hard frost and according to the results, a frost of -16°C decreased both the abundance and species richness of microarthropoda. However, no change could be measured in the populations in the summer following the treatment, which refers to a quick pace of regeneration.

In contrast with the abovementioned, weak frost or slight fluctuation around the freezing point had a positive effect on the communities. Sjursen et al. (2005) experimented with microarthropod communities in tundra habitats and found that constant cooling around -2°C increased the number of Oribatids compared to control samples. This can be explained by the fact that low temperature decreased the number of predators and favourably modified the food sources (the biomass of fungi had grown). In the same study, Sjursen showed abundance increase of the mites by a temperature fluctuation between -2°C and 2°C and explained this with the faster hatching of eggs as a consequence of frost induction. Sulkava and Huhta (2003) obtained similar results concerning both sub-zero temperature and fluctuation with samples from coniferous forests.

Another part of the experiments dealt with the effect of elevated temperature. Haimi et al. (2005) artificially increased temperature and CO_2 content in special boxes placed in Finnish coniferous forests to simulate the effects of warming. Elevated temperatures had been determined based on multiannual climatic scenarios. By increasing the temperature, no changes had been observed in the number of Oribatids as compared to

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control samples. Elevated CO_2-levels caused small changes only in the flora and microbiota, but his had not been sufficient to change the structure of the mesofauna.

Other studies dealt with polar habitats. Warming had been induced by plastic tent cover. In the following experiments, a determined number of characteristic Oribatid species had been observed. Webb et al. (1998) conducted studies on tundra heath and semi-desert soils observing 6 species. It was found that primarily aridification accompanied by warming had negative effects on the species. Mainly those species had been affected which were tipically abundant in other habitats, so they lived among suboptimal conditions in the examined sites. Webb also observed increasing abundance in case of one of the species, which can be explained by the fact that warming rendered the conditions optimal for this species. The explanation for the quick reactions given for the treatments is that polar species are not seasonal, their life-cycle is primarily influenced by temperature, thus such treatments also affect their propagation, not just their survival.

Coulson et al. (1996) had been observing consequences of tent warming on the Webb habitats for 3 years, by simulating excessive summer warming. The number of young Oribatid individuals had increased in semi-desert habitats, but no other significant change had been observed.

Hodkinson et al. (1996) applied both laboratory and field manipulations, including treatments with temperatures of 30°C and above. It was found that negative effect affecting Oribatids could be experienced only above 35°C, and time interval is an important factor in treatments around 30°C. The extent of tolerance also depends on the moisture of the soil, but it was found that warming had no strong deteriorating effect on Oribatids.

Extreme fluctuations in temperature had also been studied. Uvarov (2003) pointed out that daily temperature fluctuation of the soil affected the survival and reproduction ability of Oribatids Fluctuations with 5 and 25 °C had strong negative effects, while fluctuations with 10 and 20°C enhanced reproduction. Intermediate values had been gained by measurements on constant 15°C.

To further study the effects of temperature, several indirect experiments had been conducted on tropical areas concerning the effects of light and shade (Badejo and Akinwole, 2006). It was concluded that the structure of the mesofauna was not affected whether the sample of the habitat was taken from a spot being in shade or exposed to direct sunlight.

Changes in temperature had not directly affected Oribatids, since in most experiments applying temperature changes the mites had not reacted significantly. Even if a significant change could have been observed, the possible reason for this could have been that researchers applied harsh fluctuations and extremities, which are unexpected to take place naturally. According to these results, if fluctuations in the temperature should cause significant changes in Oribatid communities, it must be suspected that this change had not been caused directly by temperature, but indirectly, via another factor which had been affected by temperature, e. g. the quantity of food, predators or a competitor group (i. e. springtails).

Heavy metal pollutions

In the studies, the effects of the following heavy metals had been primarily examined: Zn, Cu, Cd, Ni and Pb. These examinations took place mainly in the vicinity of metallurgy facilities using heavy metals, but several studies also examined the effects

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of transport or the heavy metals released from sewage sludge used in agriculture. The first and most important question is to decide whether Oribatid mites are affected by excessive or elevated heavy metal concentrations or not. To answer this question, we must handle the different heavy metals separately. Zaitsev et al. (2001) and Skubala and Kafel (2004) found that Oribatid communities were just slightly affected by heavy metal pollution, but Migliorini et al. (2005) and Seniczak et al. (1995) pointed out that heavy metal pollution exerted serius effects on Oribatids. This difference can be explained quite simply: they measured different concentrations of hevy metals, and this also leads to a conclusion that the most „effective" heavy metal is lead. Table 1. shows the heavy metal concentrations measured by the different authors in the same units, with the lowest and highest concentrations indicated. Skubala and Kafel (2004) conducted their studies in the neighbourhood of a Zn metallurgy plant. Despite the enormous Zn concentrations, the results showed no significant decrease in density compared to the control, thus it had been concluded that Zn does not have a significant effect on this group. Similar results were obtained by Hågvar and Abrahamson (1990). Zaitsev found samples showing the greatest species richness on the most polluted sites. However these sites showed the greatest pollution in terms of zinc, copper, cadmium and lead, but the highest concentrations of Mg and Ca were also measured at these plots. According to the author, these metals had greater effects on the group than heavy metals.

It would be quite important to decide what kind of effects are exerted on Oribatid communities by heavy metal pollutions. The main question here is whether quantitative and/or qualitative effects can be expected. According to Zaitsev et al. (2001) and Seniczak et al. (1995) pollution primarily caused decrease in density, however Cortet et al. (1999), Stamou et al. (1995), Steiner (1995), Skubala and Kafel (2004) state that species richness and density were also affected. Migliorini et al. (2005) observed qualitative changes. Hågvar and Abrahamsen (1990) pointed out that although increasing lead concentration decreased species richness, there were only slight changes in total abundance because the density of several species had grown. Bargagli (1998) concluded that total abundance is far from ideal for indication purposes, since while some species vanish, others can thrive. Several studies pointed out that slight concentration of certain metals can increase the abundance of some species (Denneman and Van Straalen 1999, Hopkin et al 1985, Gackowski et al 1997, Skubala and Kafel, 2004). Consequently, Seniczak et al. (1995) classified observed species into three categories: some species were quite susceptible, others less susceptible, and some species were tolerant.

It must be mentioned that some Oribatid species are able to accumulate large quantities of metals, and great differences can be observed among species (Zaitsev and van Straalen 2001). This is because fungi can effectively take up heavy metal ions and accumulate them (Berthelsen et al., 1995; Khan et al., 2000; Valix et al., 2001, Skubala and Kafel, 2004), and as a consequence, fungivores are able to do so as well (Siepel 1995). A large part of Oribatid mites are fungivores. It could not be pointed out however that there's a correlation between the extent of accumulation and trophic level or body size (Bengtsson and Tranvik, 1989, Skubala and Kafel, 2004). These can be determined by other anatomical and physical factors (Skubala and Kafel 2004). According to the studies of Zaitsev and van Straalen (2001), microphytophagous mites are able to accumulate large amounts of zinc, but since the effect of Zn is negligible as mentioned above, it is of no significant importance in this case.

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Based on the examined studies it can be stated that lead has the greatest negative effect on Oribatids, while other metal ions (including lead as well) can even have a positive effect in low concentrations on some species. Changes are primarily qualitative, but it can not be neglected which species' abundance increased and which species vanished. Steiner (1995) suggested that other factors of the habitat of the mites must also be taken into consideration while studying the effects of heavy metal pollution: for example water content, pH etc. Beside metal ion concentrations, mainly pH had been measured in a part of the studies, but no correlations had been pointed out.

Table 1. *Range of heavy metal concentrations on sample sites from the studies of mentioned authors. Lead has been highlighted because cited authors gained uniform results for this metal: Oribatid mites has been negatively affected in all cases. Divergent results can also be explained as a consequence of different concentration ranges*

articles	Migliorini et al. 2005	Zaitsev et. al 2001	Skubala and Kafel 2004	Seniczak et al. 1995
examined areas	clay pigeon shooting range, soil samples	metallurgical plant surrounding, soil and litter samples	forests near to smelters, soil samples	forests near to smelters, samples from the trunks of pines
Zn	115-170 µg/g	58-276 µg/g	151-10454 µg/g	46-117.8 ppm
Cu	40-65 µg/g	15.1-58.6 µg/g	46.9-10.7 µg/g	21.6-843.9 ppm
Cd	-	0.01-0.92 µg/g	0.8-81.9 µg/g	2.3-7.8 ppm
Pd	80.5-1898 µg/g	9.6-54.9 µg/g	-	64.6-702.6 ppm
Ni	47.4-76.9 µg/g	13.5-43.1 µg/g	-	0.3-6.7 ppm

Organic matter content

Effect of organic matter content has been studied by the authors in two ways.

- The original organic carbon content and percentage, total N content, C/N ratio or available P content were measured (Scheu and Schultz, 1996, Kovác et al., 2001, Black et al., 2003, Osler and Murphy, 2005, Bedano et al. 2006, Salmon et al. 2006, Mitchell et al., 2007).
- Organic manure (e.g. sewage sludge) (Krogh and Pedersen, 1997, Andres, 1999, Arroyo and Iturrondobeitia, 2006) or inorganic fertilizer (e.g. ammonium-nitrate) (Moore et al., 1984, Arroyo and Iturrondobeitia, 2006, Cole et al., 2008) was added to the soil, and its effect had been examined.

Sewage sludge addition decreased the diversity (Andres, 1999, Arroyo et al., 2006) and abundance (Arroyo et al., 2006) of Oribatid mites. Theoretically an increment could have been expected, since the addition of organic matter could have had positive effects on the physical and chemical properties of the soil (Korentajer, 1991, Arroyo et al., 2006), but sewage sludge may have posed a risk to soil processes and soil-based trophic networks (Bolger and Curry, 1984, Arroyo et al., 2006).

Addition and measurement of organic compounds of any other kind gave numerous contradictory results. According to some studies, the organic matter content had no effect on Oribatid communities (Osler and Murphy, 2005). Coleman (2008) pointed out that the food network of the soil is resistant to slight changes in the source quantity. This is supported by the results of many experiments, in which the organic matter content of the soil has been enhanced with the addition of C, N and P in vain, because

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neither the abundance nor the diversity of the Oribatids changed significantly (e.g. Cepeda-Pizarro et al. 1996, Salmon et al., 2006, Cole, 2008).

Numerous studies found correlation however between the organic matter content and the structure of Oribatid communities. These works pointed out positive correlations primarily between the carbon content percentage of the soil and abundance (Kovác et al., 2001, Black et al., 2003, Bedano et al., 2006, Salmon et al., 2006) and species richness of Oribatid mites (Scheu and Schultz 1996). According to Enami et al., 1999, nitrogen content of the soil can also be determinative, in some cases even more decisive than carbon (Mitchell et al. 2007).

We compared total C% data of some studies in order to observe if there were differences of some level of magnitude in the studies giving two kinds of results. For the purpose of the analysis, we used the results of Bedano et al. (2006), and Kovác et al. (2001) from studies proving correlations, and results of Osler and Murphy, (2005) which refuted correlation. Results are shown in Table 2. The common feature of the studies is that each of them compared agricultural areas with natural habitats. We used only the average values of the original data sets. If the average value was not included in the original study, we calculated it in case of studies with data sets containing several months or plots.

Table 2. *Average C% values of soil samples from individual studies. It can be observed that in cases where correlation between the organic matter content and Oribatid communities has not been found, the difference between the areas has been far less than in cases where correlation has been indicated*

C%								
Correlation found						**Correlation not found**		
Bedano et al. (2006)				**Kovác et al. (2001)**		**Osler and Murphy (2005)**		
natural habitat	pasture	mixed	agricultural area	natural habitat	agricultural area	natural habitat	agricultural area1	agricultural area2
3.71	2.83	1.69	1.77	4.72	2.14	1.22	1.8	1.24

Further studies are needed concerning the indication of organic matter content of habitats by Oribatids. Determining total C% can be considered as a suitable method, but according to the abovementioned, it is not sufficient in some cases. Data recording by the application of fertilization is not sufficient, it would be useful to measure organic matter content also when taking animal samples. Studies are not comparable in some cases, thus it has to be emphasized that a standard method for data recording and measurements should be developed. It has to be determined that exactly which factor or component of the organic matter content of the soil has influence on the Oribatid mites.

Complex changes resulting from several factors

I. Agriculture and forestry

Agriculture

Almost all the authors examining the effects of agricultural activities on Oribatid mites pointed out that agricultural treatments affected Oribatid communities negatively: they decreased abundance and diversity (pl. Hulsman and Wolters, 1998). The reason

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for this can be approached by the changes in soil properties and the characteristics of the Oribatid mites as well.

Agricultural treatments can be various: e.g. herbicide and pesticide use, ploughing, irrigation, harvesting, and burning or collection of plant residues after harvest. These modify the properties of the soil: among others via the deterioration of the upper soil levels, drought, habitat modification and deterioration of accessibility of nutrients (Hulsmann and Wolters, 1998; Neave and Fox, 1998; Fox et al., 1999, Bedano et al., 2006). Agricultural activities decreased the amount of organic matter (Bedano et al., 2006), however, according to Osler and Murphy, (2005) decrease in the organic matter content did not correlate with the decrease in species richness. Cole et al. (2008) stated that increasing the organic matter content of the soil had not affected the diversity of the Oribatids. Grazing and trampling caused soil compaction (Bedano et al., 2006). According to the work of Bedano et al. (2006), ploughing had not affected Oribatids, which can be explained with the beneficial effect of ploughing on soil structure.

Most problems arise because plant residues are removed from the field after harvest. Numerous studies pointed out that plant coverage provides favourable conditions for soil microarthropods, since the structure of the flora has a significant effect on soil fauna through the modification of the microclimate (Gill, 1969, Berg and Pawluk, 1984, Koehler and Born 1989, Minor et al., 2004) Presence of plant residues on natural habitats decreases temperature fluctuations and moisture loss of upper soil levels, and also provides food source for the mites (Edwards and Lofty, 1975; Fox et al., 1999; Coleman et al., 2002; Koukoura et al., 2003).

Some groups of the mesofauna, e.g. Prostigmata have better tolerance against unfavourable conditions (Bedano et al., 2006). Another reason for decreasing abundance is the way of life of Oribatids. Low metabolic rate, slow development and low fecundity are such characteristics that render them unable to respond quickly to short-term, hard stresses (Behan-Pelletier, 1999). Regeneration following population decrease is also quite slow (Zaitsev et al. 2002).

Some researchers think that given their susceptibility and way of life, Oribatid mites are suitable for the indication of degradations caused by agriculture as an indicator group. However, their reliable and effective application needs lots of further studies since our methods and knowledge are incomplete. Gulvik (2007) calls attention for a line of shortcomings, for example the lack of standardization of sampling and data processing methods.

This is most obvious and urgent in studies concerning land use. An expected requirement of an indicator group is that presence and quantity of species in the samples taken from a given area should indicate some kind of phenomenon. To reach this goal however, the studies and examinations should be carried out in a way that we would be able to give an exact description of phenomena, and link the two factors together based on the present Oribatid community. Information recording though, is quite inaccurate in land use studies. Life conditions of soil Oribatids are mainly determined by the properties of the soil and agricultural activities modify these circumstances. When a natural and a cultivated area are compared, mostly the organic matter content, pH and moisture of the soil are recorded beside flora (Bedano et al., 2006, Minor et al. 2004, Osler and Murphy, 2005). In most cases it turns out however, that there's no correlation among measured soil properties and observed characteristics of the community (Minor et al., 2004, Bedano et al., 2006), or just in case of organic matter content at best, but contradictions also can be found here (see „Organic matter content"). In many cases, not

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even the agricultural treatment or tillage has been properly described, e.g. „low-input", „high-input", „conventional agricultural practices"; but these are considered as a basis for comparison (Bedano et al., 2006, Osler et al. 2008).

Another problem is how we can use Oribatid communities. There are some authors who took only abundance into consideration (e.g. Bedano et al. 2006). We have some information that some groups are susceptible or tolerant to agricultural activites (Gulvik, 2007). The species Tectocepheus velatus, and the groups of Nothroidea and Ptyctimia are tolerant (Gulvik, 2007). This is quite insufficient however to gain exact information in order to describe a site. The aim of studies conducted these days should be to remedy these problems, but suitable methods are required to achieve this goal. Gulvik (2007) calls for international standards in the phases of sampling, data processing and taxonomical processing. It should be clearly defined what agricultural activities cause which kind of changes in the soil, and which are those among these effects, that actually affect mite commuities

Forestry

Behan-Pelletier (1999) emphasized that the most abundant and diverse group of soil mesofauna were the Oribatids even in forest habitats. Changes in this community can have an important indication role. According to Behan-Pelletier (1999) the density of groups with parthenogenetic reproduction increased following disturbation, which could cause changes in the structure of the community. Several studies tried to determine the changes in Oribatid communities caused by disturbations in forest habitats. Numerous types of disturbations have been examined in forests but these studies are hard to compare and as a consequence, obtained various results.

Lindo and Visser (2004) studied the effect of different extents of forest clearing. Partial forest clearing had milder effect than clear-felling, but the change could have been observed only at the level of abundance. In some cases diversity indices and uniformity had also grown due to disturbance, which could be explained with the abundance loss of dominant species. Change in abundance is related to organic matter content, litter input and microbial biomass - (Marra and Edmonds, 1998, Lindo and Visser, 2004), namely the reduction of available nutrients (Battigelli 2004, Lindo and Visser, 2004). Besides, the microclimate of the soil changes as well (Marra and Edmonds, 1998), the size of soil pores decreases, which means the soil compacts (Battigelli et al. 2004) due to forest clearing. Battigelli et al. (2004) studied the effect of soil compaction and organic matter decrease following forest clearing. It was found that these interventions modified species richness beside decreasing the density of species, and thus caused changes in the structure of the community. The difference between the results of the two studies could have been caused by the difference in the applied methods, since Lindo and Visser (2004) studied the mesofauna of the sites as late as 2.5 years following forest clearings.

While Battigelli et al (2004) observed significant decrease in density and number of species when organic matter (soil with roots) had been removed from the area, Berch et al (2007) did not point out significant difference comparing control and treated sites, where the upper layer of the soil had been removed. It has to be mentioned however, that the study of Berch et al (2007) took place 5 years following the treatment. Fire also removes the organic matter from the upper layers of the soil, but the work of Berch et al (2007) did not show changes in the Oribatid communities. Burning treatments gave

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contradictory results in most cases, which could be attributed again to the differences in the treatments.

Maraun et al. (2003) studied the physical disturbance of the soil, when the soil had been sieved at regular intervals. The results of this study are hardly comparable to that of the former mentioned works, since this treatment is hardly reconcilable with the actually occurring disturbances like forest clearing, decrease in organic matter content or soil compaction. Maraun et al. (2003) however, in the wake of the results of Behan-Pelletier (1999) found that groups with parthenogenetic reproduction (Tectocepheus sp., Oppiidae) were tolerant to disturbances. In the work of Lindo and Visser (2004), the proportion of species with parthenogenetic reproduction did not differed from that of the control sites. Changes expected in case of species with asexual reproduction could not be showed by Berch et al. (2007) as well.

Interventions taking place in forests can induce multiple changes in Oribatid communities, however the exact effects of these changes are not yet properly described. According to Lindo and Visser (2004) forest cleraing can induce only quantitative, and not qualitative changes, thus the possibility of using Oribatids as indicators is quite limited. In case of other types of disturbances however, even the species structure has been changed (Berch et al., 2007, Battigelli et al., 2004, Maraun el at., 2003). Comparability of results needs methodological standardizations, just as Gulvik (2007) has recommended when studying the effects of agricultural activities.

If the observation of the effects of forest clearings is demanded, then sampling must be done in periods immediately both before and after the interventions (forest clearing, soil dumping, soil layer removal etc.), since circumstances in control areas are not necessarily identical to those present in the period before the disturbance. Apart from this, attention should also be paid for that the process of regeneration should not be studied by collection of samples just in a single period. Constant monitoring is needed begining from the implementation of the treatment in order to exactly observe the changes.

II. Comparison of natural habitats

These examinations try to explore what properties of habitats play a role in pattern generation, among which spatial and temporal changes can be distinguished. Observations on seasonality have not yielded considerable results (Reynolds et al., 2003, Noti et al., 1996, Badejo et al., 2002, Moldenke and Thies, 1996). Habitats and sampling frequencies are quite different and hardly comparable. Currently we do not possess any satisfying results on seasonal dynamics. A number of studies (e.g. Reynolds et al., 2003) surveying temporal changes measured the total abundance of the community. Measuring the changes in the number of individuals of larger groups does not mean thorough examination. It is worth to survey the temporal structures of the entire community on such places where seasons are well discernible. One of the most important studies has been made by Irmler (2006), who studied the seasonal changes of an Oribatid community living in the OL and OF layer of a beech forest. It was found that only the annual mean temperature had significant effect on the structure of the community. The study yielded more results when Irmler surveyed the seasonal dynamics of individual species. Mainly the amount of precipitation affected the abundance of certain species, but some species had been affected more significantly by temperature (primarily by the mean temperature in January). The significance of

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species-level examination was confirmed by the fact that certain species reacted differently on the surveyed parameters.

Spatial comparisons applied different scales; part of them compared soil and foliage of forests. These studies revealed that Oribatids of the soil showed greater α-diversity and species richness, but β-diversity proved to be greater in the foliage, which means difference among samples taken from individual trees has been greater than that of the samples collected from the soil (Lindo and Winchster, 2006, Fagan et al. 2006).

Comparison of elevations above sea level attracted great attention: primarily the abundance and species richness of Oribatids have been studied in zones of different altitudes. However, obtained data are not concordant: according to Migliorini and Bernini, (1999) and Fagan et al., (2006) the abundance of Oribatids decreased with altitude, but Jing et al., (2005) and Reynolds et al., (2003) observed an opposing tendency. Fagan et al., (2006) pointed out a decrease in species richness, Migliorini and Bernini, (1999) observed a growth in diversity as a function of increasing altitude. It has to be mentioned by these contradictory results that altitudes of sampling and habitats are hardly comparable, and even if they were, this would not guarantee consistent results. This has been pointed out by Andrew et al (2003) in an extended series of studies conducted on different altitudes in Australia and New Zealand.

Beside altitude, vegetation also changes greatly when progressing upwards on a hill. Studies mentioned above did not lay an emphasis on vegetation. The work of Balogh et al.(2008) however demonstrates altitude as a difference in the type of vegetation: rainforest, moss forest and paramo. Samples were taken from the mountains of Brazil, Costa-Rica and New-Guinea. This work showed that the structure of Oribatid mite communities was primarily determined by the type of vegetation and not by the distance of several thousand kilometres, which means that climate and ecological conditions have stronger effects than zoogeographical connections (Balogh et al., 2008).

Abundance, species richness and diversity - summary

Studies examining Oribatid communities almost always measure which Oribatid species and in what quantity are present in samples taken from the given area. Species composition, abundance, total abundance, species richness, diversity and the uniformity of the community can be calculated from these data. In most cases, changes in the communities are examined using these variables.

When given the same climate, abundance, species richness and diversity of the Oribatids are greater in natural areas (forest or habitats not strongly affected by human activity) than in areas affected by agriculture (e.g. plant production or animal husbandry) or forestry (e.g. clear-felling, burning etc.) (Bedano et al. 2006, Osler et al., 2006, Cole 2008, Olejniczak 2004, Arroyo and Iturrondobeitia, 2006, Migliorini et al., 2003, Altesor et al., 2006). The observation of Bedano et al. (2006) can be mentioned as an exception: it was found that the abundance of pastures was higher than that of natural forests.

Decrease in abundance can be caused by hard frost (Sulkava and Huhta, 2003) and serious heavy metal pollution (Seniczak et al., 1995). According to Osler et al. (2006), mainly the number of individuals is lower in the initial state of succession. Decrease in abundance could be pointed out primarily as a result of water deficiency (O'Lear and Blair, 1999, Lindberg et al., 2002), but contradictory results had been also obtained (O'Lear and Blair, 1999, Melamud et al., 2007). Lindberg and Bengtsson, (2006)

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showed that community regeneration following drought can not be satisfactorily measured by the sole application of total abundance. Decrease in the abundance of Oribatids can also be caused by ash treatment of sour, acidic soils (Liiri et al., 2002). In Japanese coniferous forests it has been shown that the abundance of Oribatids was greater in mixed litter (litter of several tree species) than in litters consisting of only one tree species (Kaneko and Salamanca, 1999). Kovác et al., (2001) explored positive correlation between the nutrient content of the soil and abundance, but it was contradicted by several other studies (e.g. Osler and Murphy, 2005).

Removal of winter snow cover lead to a decrease in species richness, since the mesofauna of the soil has been exposed to greater fluctuation of temperature (Sulkava and Huhta, 2003). Response of species to heavy metal pollution varied greatly, sometimes even moderate pollution resulted in the highest species richness (Skubala and Kafel 2004). Drought generally decreased species richness (Tsiafouli et al., 2005), but there were several examples for growth as well (Melamud et al., 2007). Ash treatment lowered abundance and also species richness (Liiri et al., 2002). In mixed litter, both species richness and abundance were higher (Kaneko and Salamanca, 1999). Fagan et al., (2006) found in Canadian coniferous forests that species richness of Oribatids in the soil had been greater when comparing Oribatid communities of the foliage and soil.

Table 3. *Strongly abridged summary of information from studies on characteristics of Oribatid communities. (↑=increases or greater; ↓=decreases or lower)*

abundance	**species richness**	**diversity**
artificial disturbance↓	artificial disturbance↓	artificial disturbance↓
hard frost↓	snow cover removal↓	irrigation↑
drought↓	drought↓	drought↓
early stage of succession ↓	ash↓	number of ecotones↑
diverse litter mix↑	diverse litter mix↑	diverse litter mix↑
ash treatment↓		soil > foliage
heavy metal pollution↓		
organic mater content↑		

Diversity data can be found primarily in agricultural and forestry studies. It has been pointed out that irrigation (enhancing the moisture content of the soil) increased the diversity of Oribatid communities, because it raised the individual numbers of rare species (Tsiafouli et al., 2005). Drought had a detrimental effect on diversity (Lindberg et al., 2002). Studies of Taylor and Wolters (2005) pointed out that Oribatid diversity had been greater in a more decomposed beech litter than in fresh litter. Seniczak et al., (2006) concluded that Oribatid diversity can be increased by increasing the number of

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ponds of forest habitats, since this means more ecotones and leads to the presence of such kind of species which prefer humid habitats and are normally absent from forest habitats. Age of temperate deciduous forests did not affect diversity (Erdman et al., 2006). Growth in the diversity of tree species did not increase the diversity of Oribatids living in the soil of these forests (Kaneko and Salamanca, 2005). However, growing diversity of the litter not only increased abundance and species richness, but diversity as well (Coleman 2008). (*Table 3.*)

Conclusion and outlook

With the overview of available studies, it can be clearly explored how various characteristics of Oribatid communities are modified due to changes in moisture, temperature, heavy metal concentration, organic matter content and level of disturbance. The most important question concerning the application of Oribatids as indicators is to clarify what kind of information content do natural Oribatid coenological patterns possess from the aspect of bioindication. Most of the variables listed above can be directly measured, since rapid methods are available to quantify temperature, heavy metal content etc. of the soil. Responses of Oribatids are worth to study in a more complex approach. Even now we have an expansive (but far from satisfactory) knowledge on how communities change due to modifications of different factors. These pieces of information necessitate the elaboration of such methods which render Oribatid communities suitable for the task to prognosticate what extent the given site can be considered near-natural or degraded, based on the Oribatid composition of a single sample taken from the given area. Raising further questions will be possible only after obtaining the answer for this problem. However, answering this problem needs extensive and coordinated work: approriate reference sites need to be appointed to clarify the concept of naturality, sampling and processing methods need to be standardized internationally – in conformity with the given environmental conditions – and the field of data processing methods also has to be developed. Definition and testing of Oribatid-based (or mesofauna-based in a broader sense) coenological indicators are also undoubtedly needed. The usefulness of Oribatid characteristics summarized in the introduction had been recognized long ago, now it is time to conduct research in a way that enables to explore and exploit the actual advantages Oribatid mites provide.

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