COMPARATIVE EVALUATION OF BIOLOGICAL METHODS TO SUPPORT DECISION-MAKING FOR BIODEGRADATION-BASED SOIL REMEDIATION

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ABSTRACT

Management of a contaminated site in practice includes site-specific risk assessment as well as selection, evaluation and establishment of the remediation technologies. In case of biodegradation-based soil remediation reliable methods for monitoring and assessing soil microflora and its activity, microcosms testing of site-specific biodegradation of the pollutant are a prerequisite for a successful remediation project. Soil biological investigations, such as measurements of soil respiration, enzyme activities, microbial counts and specialized contaminant-degrading cell concentration can give information about the presence of viable microorganisms, on the metabolic activity of soil and on the impact of the effects of environmental stresses, such as hydrocarbon contamination.

The aims of this research were to a) to evaluate several biological soil testing methods as a toolbox for characterizing the biodegradation processes in soil and b) to test the usefulness of an chemical method (cyclodextrin-extraction technique) for assessing the biologically available fraction of contaminants.

Lab-scale microcosm experiments with diesel and transformer oil spiked soils were performed and evaluated by chemical analyses and biological methods presenting different aspect of soil microbial characteristics. As part of the biological methodology 1) modified Most Probable Number method for estimation of contaminant degrading cell concentration, 2) soil dehydrogenase enzyme activity test, 3) soil respiration test in a “closed bottle”, and 4) soil respiration method in a self-designed flow-through system (dynamic soil-reactors) were applied and studied in this research. The Extractable Petroleum Hydrocarbon (EPH) content was analysed from the hexane-acetone extract of the soils by gas chromatography (GC-FID). Aqueous cyclodextrin solutions were applied for the extraction of organic contaminants from soil to estimate their bioavailability.

Biodegradation could be monitored well by all of the investigated soil biological parameters. The biological toolbox used to characterize biodegradation processes in soil has provided a meaningful characterization of the microbial populations and activities associated with contamination. The statistical analyses by the use of STATISTICA showed very good correlation between the results of RAMEB-extractions (REH) and the results of dehydrogenase enzyme activity (DEH) and the soil respiration measured in “closed bottle” (RESP CB) in case of diesel oil. A good correlation was also obtained with the HPBCD extracted hydrocarbon content (HEH), the solvent extractable hydrocarbon content (EPH) and the concentration of the oil-degrading cells (MPN). In case of transformer oil contaminated soil the HPBCD extracted hydrocarbon content (HEH) strongly correlated to the transformer oil-degrading cell concentration (MPN), and also with the soil respiration measured in “closed bottle” (RESP CB). These results suggested that the use of cyclodextrin-extraction can be suitable for prediction of hydrocarbon degradation in soil.

The biological toolbox together with chemical methods provided valuable information about the bioavailability of the contaminants, biodegradation processes and evidence of natural attenuation.

INTRODUCTION

Appropriate site characterization performed in a timely and cost efficient manner is an essential step towards sustainable site remediation of contaminated sites. However site specific-risk assessment usually does not comprise test and methods supporting technology selection and design. Taking into consideration that a sustainable remediation technology application is site-specific, cost-efficient pre-implementation methods providing specific information are necessary throughout the site characterization process in order to make correct decision and to ensure an optimal performance of the remediation actions.

An integrated approach, complementary biological and ecotoxicological testing to the chemical analysis should be used for the characterization of processes in the contaminated soil (Frische and Höper, 2003; Gruiz, 2003; Gruiz, 2005; Gruiz et al, 2008; Leitgib et al, 2003; Molnár et al, 2003; Molnár et al, 2007). Soil testing TRIAD approach, proposed by Gruiz et al. (2005) is necessary in environmental assessment and monitoring, included the monitoring of contaminated sites, natural attenuation and remediation technologies. It means that physico-chemical analyses should be accomplished by biological measurements and ecotoxicity testing to get additional information about bioavailability, biodegradability of the contaminants, about activity and adaptive behaviour of the soil biota, about not measured or not measurable but dangerous components.

According to Critto et al. (2007) a decision support system based on the TRIAD scheme is necessary to support experts and decision makers in selecting a suitable set of measurement endpoints to be applied at the case study and to aggregate the results of the selected measurement endpoints into integrated risk indexes. These above mentioned measurement endpoints belong to three major groups: environmental chemistry (e.g. chemical-physical properties and bioavailability of pollutants), ecotoxicology (e.g. bioassays) and ecology (e.g. species abundance or richness).

Biological methods support decision-making and can be useful from selection and design of the treatment technology through the biodegradation based remediation process until the monitoring of the site. To assess whether a biological treatment of the contaminated soil is appropriate, every site relating to its microbial populations and the bioavailability, and biodegradability of its contaminants should be characterized. The biological answer integrates all the effects and interactions between contaminants, soil and biota, which can hardly be predict by using a chemical model (Frische and Höper, 2003; Gruiz, 2003; Gruiz, 2005). Microbiological characterization of contaminated soils (biomass, soil respiration, enzyme activities, specialized contaminant degrading cell concentration etc.) supports information on the physiological state and the activity of the soil microflora or whether microorganisms do exist in the contaminated soil. The most widely used biological methods for assessing the effects of pollutants on the metabolic activity in the soil, for characterization of biodegradation of the contaminants and for monitoring feasibility of bioremediation are: soil microbial counts, soil respiration and dehydrogenase activity (Balba et al, 1998; Frische and Höper, 2003; Margesin et al, 2000; Sabaté et al, 2004; Szécsényi-Nagy et al, 2003).

The success of bioremediation of contaminated soils is determined by the bioavailability of the contaminants. The use cyclodextrins (CDs) for the intensification of bioremediation by improving the mobility and bioavailability of contaminants has recently been studied. Many publications demonstrated that CDs, can be useful in physical and chemical treatment technologies (McCray & Brusseau 1998; McCray & Brusseau 1999; Sheremata & Hawari 2000; Ko et al. 2000) as well as in soil and wastewater bioremediation due to their solubilising effects (Bardi et al. 2000; Fava et al, 1998; Gruiz et al. 1996; Oláh et al, 1988). The biodegradation of the highly persistent polychlorinated biphenyls (PCBs) was significantly enhanced in the presence of randomly methylated beta-cyclodextrin (Fava & Ciccotosto 2002; Fava et al, 2002; Fava et al, 2003). RAMEB enhanced the biodegradation also of the recalcitrant mazout (distillation residue of oil refinery) in a historically contaminated soil (Molnár et al, 2003; Molnár et al, 2005). Randomly methylated beta-cyclodextrin (RAMEB) was found to be slowly biodegradable by the indigenous microflora of contaminated soils, displaying about 1-year half-life time (Fenyvesi et al. 2002). The applicability of an aqueous solution of hydroxypropyl beta-cyclodextrin (HPBCD) to predict the microbial available fraction of mono- and polycyclic aromatic hydrocarbons has been demonstrated in the few years past (Allan et al, 2006; Reid et al, 1998; Reid et al, 2000). The cyclodextrins change the availability of organic contaminants, the HDBCD and RAMEB can decrease the octanol-water partition coefficient (Kow) of various organic compounds (Szaniszló et al, 2005).

The aims of this study were firstly the evaluation of several biological methods with regard to their usefulness for monitoring and characterizing microbial activities and biodegradation in diesel and transformer oil spiked soils. Secondly, the suitability of the cyclodextrin-extraction technique using aqueous CD solutions to estimate the bioavailability of organic contaminants in soil was assessed.

MATERIALS AND METHODS

Small-scale 6-week-long laboratory experiments with hydrocarbons-contaminated soils were performed for studying the bioavailability and biodegradation of hydrocarbons and to evaluate the applied complex biological-chemical methodology.

Experimental setup of microcosms experiments

Contaminated soils with hydrocarbons were investigated in multispecies microcosm systems. Microcosm experiments were performed with three-phase artificially contaminated soil in static and dynamic reactors. Garden soil was spiked with diesel oil and transformer oil in the concentration of 30,000 mg/kg. The uncontaminated garden soil was used as a control for studying the effects of contaminants. The soils were amended with inorganic nutrients ($(NH_4)_2SO_4$, KNO_3, KH_2PO_4) to reach the final C:N:P ratio of 100:10:1 and were incubated at 25±2 ^oC. Biodegradation experiments in static and dynamic soil microcosms were started after 4 weeks adaptation period after spiking the soil.

Dynamic soil-reactors

Three self-designed flow-through 1 dm^3 of volume column-reactors filled with 500–500 g of soils were used in dynamic microcosms experiments at 25±2 ^oC. The reactors filled with the control (uncontaminated) and contaminated soils were aerated 2 hours daily with the 10 l $air{*}h^{-1}$ of aeration rate. Two traps filled with NaOH ensured the CO_2-free atmospheric air. The CO_2-free air was passed through the soil-columns. The CO_2 produced by the soil microorganisms during aeration was trapped in 1 N NaOH solution and measured by HCl-titration. Optimal humidity (10–15 % $^w/_w$) was maintained throughout the whole experiment.
The soil respiration, CO_2 production of microbes in dynamic aerated reactors (RES AER) indicates the effect of contamination, the adaptive potential, the activity of soil microorganisms. This system can be used by measuring CO_2 production for characterization of microbial activities in soil, for testing biodegradability or toxic effect of organic contaminants. Also the effect of technological parameters and additives can be investigated (Gruiz, 2005; Leitgib et al, 2007; Molnár et al, 2007).

Static soil-reactors

Three 5 dm^3 of volume static reactors were set up for carrying out static microcosms experiments with 2–2 kg of diesel and transformer oil contaminated soil and uncontaminated (control) soil respectively. The reactors were incubated under aerobic conditions at 25±2 ^oC. At intervals between weeks 0 and 6, soil samples from each microcosm were taken and analysed after sampling at various points, combining and mixing. Biodegradation and the bioavailability of the contaminants and the activity of the indigenous soil microorganisms were characterized by chemical and biological methods at start, after 2 week, 4 and 6 weeks.

The integrated biological-chemical methodology

Biological and chemical methods were used for determination of the microbial activity, for characterization of the biodegradation processes and the bioavailability of the contaminants in the soil. As the elements of the biological toolbox 1) modified Most Probable Number method for estimation of contaminant degrading cell concentration, 2) soil dehydrogenase enzyme activity test, 3) soil respiration test in a “closed bottle”, and 4) soil respiration method in a self-designed flow-through system (dynamic soil-reactors, see above) were applied and studied in this research.

A *most probable number (MPN) procedure* was developed to estimate the population density of the *specialized hydrocarbon-degrading cells*. For growing the hydrocarbon-degrading cells a dilution series of contaminated soils were used in three replicates, containing organic contaminant (diesel and transformer oil) as the only carbon source.

The applied liquid medium was supplemented with inorganic salt solution, trace elements and with an artificial electron acceptor of the 2-(p-iodophenyl)–3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT). The Most Probable Number (MPN) was calculated from the red colour (+/-) by using probability tables (Lorch et al. 1995).

The assay of dehydrogenase can be used as a simple method to examine soil microbial activities and possible inhibitory effect of the contaminants on the soil microorganisms (Balba et al, 1998). Nearly all microorganism reduce 2,3,5-triphenyl-tetrazolium-chloride (TTC) as an artificial electron acceptor to triphenyl formazan (TPF), which can be colorimetrically measured. *Dehydrogenase enzyme activity* (DEH) based on the estimation of the TTC reduction rate to TPF was characterized and used as an index for overall microbial activity of the soil according to Alef (Alef, 1995a). Field-moist soil (5 g) was incubated for 24 h at 28 ^{o}C in TTC-Tris-HCl buffer solution. TPF concentration was colorimetrically measured after extraction with acetone at 546 nm. For interpretation of the dehydrogenase activity results µ TPF/g soil was calculated.

Estimation of soil respiration one of the oldest and still the most frequently used method for quantifying microbial activities in soils. Soil respiration can be determined by using simple techniques by measuring the CO_2 production or O_2 consumption (Alef, 1995b). *Soil respiration in a "closed bottle*" (RES CB) was characterized and evaluated by Sensomat System. The Sensomat System one of the most advanced manometric (respirometric) measurement system. It's suitable for a great range of environmental monitoring, such as soil respiration, measurement of biological degradability of contaminants etc. A vial containing NaOH is placed in each vessel to trap CO_2. So if oxygen is consumed in the closed vessels at a constant temperature negative pressure develops. Pressure difference (decrease) due to microbial activity in closed 500 ml of volume vessels filled with 100 g of moist soils was measured by the Sensor-IR for 5 days at 25 ^{o}C in Sensomat System. For interpretation of the results the linear part of the respiration curve (pressure–time) was used. From the linear part of the curve *respiration-index* (RI) was determined by linear regression.

The results of the applied biological toolbox were complemented by chemical and ecotoxicological analyses of contaminated soil samples throughout the microcosm experiments.

We carried out mortality test with the Collembolans (*Folsomia candida*), commonly known as springtails before starting the experiment and after 6 weeks. Springtails are the most numerous and widely occurring insects in terrestrial ecosystems. Microarthropods as e.g. springtails are said to have an important function regarding the maintenance of soil functions. Due to their short life cycles, high number of species and their high density, the important requirement for using them as indicator organisms is fulfilled. Ten *F. candida* specimens of twenty-days-old springtails from a synchronized culture were transferred into the test flasks, containing different dilutions of contaminated soil and reference OECD soil. Test flasks were incubated at 20±2 °C in the dark for 7 days. At the end of the incubation period, each soil in the test flask was flooded with distilled water and the floating, living animals were evaluated by counting. The endpoints used for the animal tests were LD_{20} or LD_{50} values, where LD_{20} and LD_{50} mean soil doses that caused 20 % and 50 % lethality. LD_{20} and LD_{50} values were determined from dose-response curve (inhibition percent values of different dilutions) after sigmoidal fitting of data by ORIGIN 6.0 software.

Solvent Extractable organic Material (SEM) content was measured after hexane-acetone (2:1) extraction by gravimetry (HS 21470-94, 2001). The so-called Extractable Petroleum Hydrocarbon (EPH) content was analysed from the same extract by gas chromatography with flame ionisation detector (GC-FID) according to the Hungarian Standard (HS 21470-94, 2001).

Aqueous cyclodextrin solutions (10 %) were applied for the ultrasonic extraction of organic contaminants from soil to estimate their bioavailability. Cyclodextrin derivatives of high solubility and high solubilizing potential such as randomly methylated beta-cyclodextrin (RAMEB, Wacker Chemie, Munich, Germany) and hydroxypropyl beta-cyclodextrin (HPBCD, Wacker Chemie, Munich, Germany) were used and compared. The cyclodextrin extracts were transferred into hexane-acetone after solid-phase extraction and analysed by gas chromatography.

The results of the non-exhaustive cyclodextrin extraction were compared with the results of the exhaustive extraction by organic solvents and those of several biological and ecotoxicological tests characterizing the bioavailability of the contaminants.

Data evaluation of tested parameters was processed by statistical analyses. Data evaluation of small-scale laboratory experiment series was processed by correlation analyses by the use of StatSoft® Statistica 6 program.

RESULTS AND DISCUSSION

Increased soil microbiological activities (such as soil respirations, dehydrogenase enzyme activity and specialized contaminant degrading cell concentration) in oil-polluted soils were attributed to the adaptation of the hydrocarbon-degraders and gave information on the presence of viable microorganisms as well as on biodegradation processes in the soil. All of the applied biological soil testing methods showed higher microbial activity in diesel oil contaminated soil than in transformer oil contaminated soil proving the good adaptation of soil microorganisms to biodegradable contaminant. The biological toolbox including simple and cost-efficient microbiological soil testing methods was found to be a reliable indicator of hydrocarbon biodegradation the soil.

Soil respiration in dynamic aerated system (RESP AER)

In aerated soil reactors the microbial activity determined by CO_2 production of microorganisms was continuously higher at the diesel oil contaminated soil (Figure 1.). The CO_2 production showed decreasing trend in the first two weeks later slightly increased soil respiration was found. The measured CO_2 production is the resultant of contrary procedures: an increase due to mobilisation and a decrease due to biodegradation. After consumption of the easily biodegradable fractions of the hydrocarbons “further” adaptation period was necessary for microbes to degrade the residual hydrocarbons in soils.

Specialized hydrocarbon-degrading cell concentration (MPN)

The number of specialised oil-degrading cells decreased continuously with the consumption of the contaminant both in diesel and transformer oil contaminated soils (Figure 2.). The results indicated that soil microorganisms adapted to the hydrocarbon contamination in the 4-weeks of incubation period. After starting the experiment the decreasing number of the oil-utilizing bacteria corresponded to the decreasing EPH content (Figure 6.).

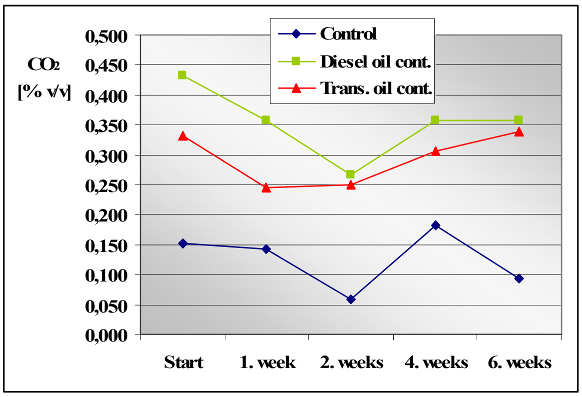

Figure 1. CO_2 production in dynamic aerated reactors

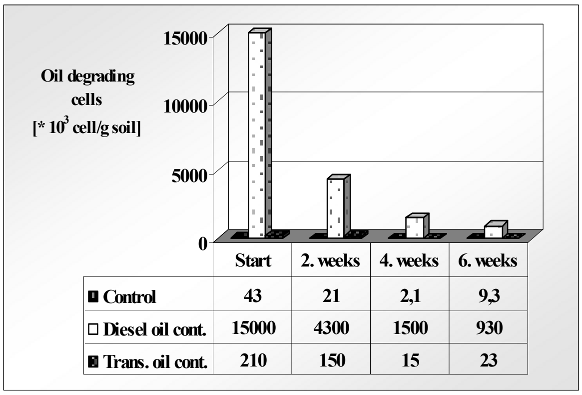

Figure 2. Changes of oil-degrading cell-concentration in static reactors

Soil respiration in “closed bottle” (RESP CB)

The results of soil respiration measured in closed bottle system indicated also the existing hydrocarbon-degrading activity in the soil, and the adaptive behaviour of the soil (Figure 3.). The slight increase of the respiration index in the first period of the experiment showed the increased availability of contaminants and the higher microbial activity.

Dehydrogenase enzyme activity (DEH)

Like all soil biological parameters dehydrogenase enzyme activity was significantly higher in the diesel oil contaminated soil (Figure 4.). After an initial enhancement of the enzyme activity subsequent decline was observed in the diesel oil contaminated soil. In case of transformer oil contaminated soil the dehydrogenase activity decreased in the first period, after 4 weeks a slight increase was obtained.

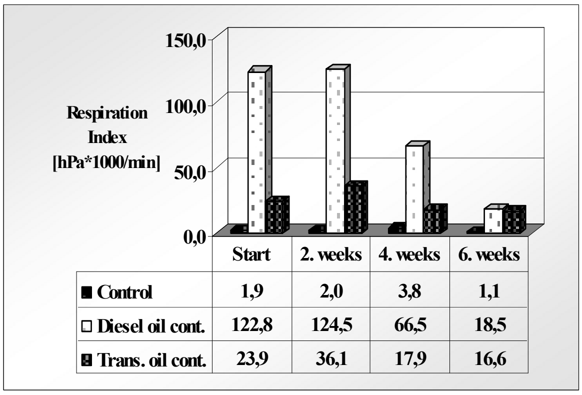

Figure 3. Respiration Index in static reactors measured by “closed bottle”test

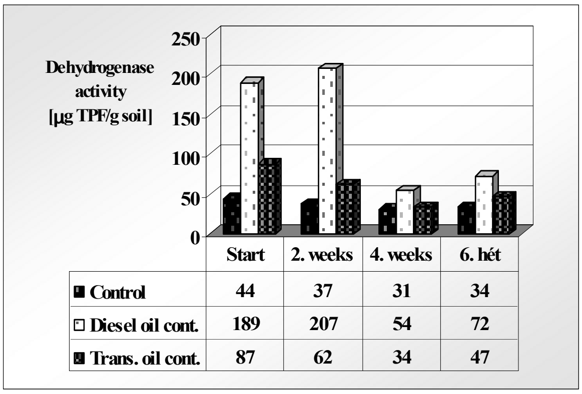

Figure 4. Changes of dehydrogenase activity in static reactors

Soil toxicity measured by Folsomia candida mortality test

Toxic effect of soil samples was measured at start and after 6 weeks. Soil toxicity measured by *Folsomia candida* mortality test decreased in case oil diesel oil contaminated soil, but slightly increased in case of transformer oil contaminated soil (Table 1.).

Table 1. Soil toxicity measured by *Folsomia candida* mortality test

Soil	*Folsomia candida* mortality test				
	Start		*After 6 weeks*		
	LD_{50} [g soil]	*Interpretation*	*LD_{50} [g soil]*	*Interpretation*	*Decreaase in toxicity [%]*
Control soil	-	non toxic	-	non toxic	
Diesel oil contaminated soil	8.8	toxic	14.3	toxic	38 % (decrease)
Transformer oil contaminated soil	9.1	toxic	8.6	toxic	- 5 % (increase)

- no inhibition was found

Solvent Extractable organic Material (SEM) and Extractable Petroleum Hydrocarbons (EPH)

The measured solvent extractable material (SEM) content and the content of the extractable petroleum hydrocarbons (EPH) showed continuously decreasing tendency during the experiment (Figure 5–6.) due to the activity of the adapted soil microbes.

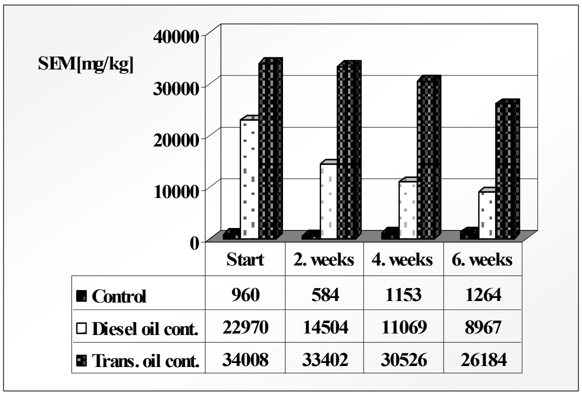

Figure 5. Changes of Solvent Extractable organic Material in static reactors

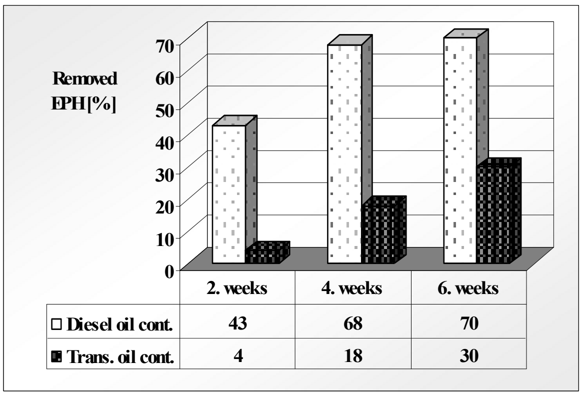

Figure 6. Hydrocarbons-removal in static reactors during the 6-week experiment

Cyclodextrin Extractable Hydrocarbons (REH, HEH)

Aqueous cyclodextrin solutions of RAMEB and HPBCD were applied to test the usefulness of a chemical method (cyclodextrin-extraction technique) for assessing the biologically available fraction of contaminants (Figure 7–8). Gas chromatographic analysis of the cyclodextrin-extracts showed that the compounds in higher concentration and in a wider molecular weight region were extracted by RAMEB than by HPBCD.

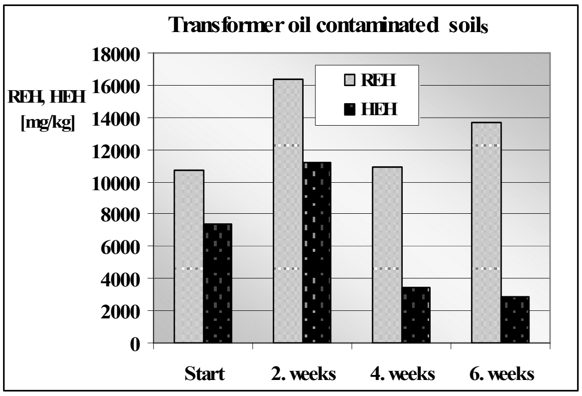

Figure 7. Changes of cyclodextrin extractable hydrocarbons (REH, HEH) in transformer oil contaminated soils

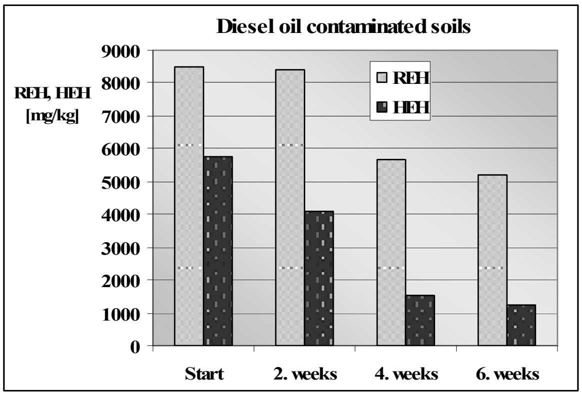

Figure 8. Changes of cyclodextrin extractable hydrocarbons (REH, HEH) in diesel oil contaminated soils

Gas chromatographic analysis of cyclodextrin-extracts also demonstrated that the components in the lower molecular weight region are extracted by cyclodextrin solutions compared to the exhaustive extraction by organic solvents.

The results of cyclodextrin-extraction were compared with those of several chemical and biological methods by correlation analyses using StatSoft® Statistica 6 program (Table 2–3.).

Table 2. Correlation matrix between biological and chemical properties of the diesel oil contaminated soil

Correlation matrix (coefficients and significance levels (p)) between biological and chemical properties of the diesel oil contaminated soil								
	EPH	***REH***	***HEH***	***SEM***	***MPN***	***DEH***	***RESP CB***	***RESP AER***
REH	0,8516	1,0000						
	p=0,148	p= ---						
HEH	**0,9689**	**0,9546**	1,0000					
	p=0,031	p=0,045	p= ---					
SEM	**0,9927**	0,8380	**0,9568**	1,0000				
	p=0,007	p=0,162	p=0,043	p= ---				
MPN	**0,9809**	0,9369	**0,9985**	**0,9687**	1,0000			
	p=0,019	P=0,063	p=0,002	p=0,031	p= ---			
DEH	0,7836	**0,9727**	0,9060	0,7438	0,8855	1,0000		
	p=0,216	p=0,027	p=0,094	p=0,256	p=0,114	p= ---		
RESP CB	0,7799	**0,9590**	0,8910	0,7991	0,8687	0,8796	1,0000	
	p=0,220	p=0,041	p=0,109	p=0,201	p=0,131	p=0,120	p= ---	
RESP AER	0,4856	-0,0388	0,2555	0,5109	0,3065	-0,1523	-0,0686	1,0000
	p=0,514	p=0,961	p=0,744	p=0,489	p=0,693	p=0,848	p=0,931	p= ---

Bold values (marked correlations) are significant at p < 0,05000

Table 3. Correlation matrix between biological and chemical properties of the transformer oil contaminated soil

Correlation matrix (coefficients and significance levels (p)) between biological and chemical properties of the transformer oil contaminated soil								
	EPH	***REH***	***HEH***	***SEM***	***MPN***	***DEH***	***RESP CB***	***RESP AER***
REH	-0,0177	1,0000						
	p=0,982	p= ---						
HEH	0,8617	0,4913	1,0000					
	p=0,138	p=0,509	p= ---					
SEM	**0,9868**	-0,0378	0,8354	1,0000				
	p=0,013	p=0,962	p=0,165	p= ---				
MPN	0,9018	0,3431	**0,9659**	0,8440	1,0000			
	p=0,098	p=0,657	p=0,034	p=0,156	p= ---			
DEH	0,8641	-0,3057	0,6091	0,7925	0,7737	1,0000		
	p=0,136	p=0,694	p=0,391	p=0,207	p=0,226	P= ---		
RESP CB	0,7109	0,6808	**0,9606**	0,7062	0,8620	0,3647	1,0000	
	p=0,289	p=0,319	p=0,039	p=0,294	p=0,138	P=0,635	p= ---	
RESP AER	-0,4428	-0,6827	-0,7152	-0,5148	-0,5100	0,0530	-0,8607	1,0000
	p=0,557	p=0,317	p=0,285	p=0,485	p=0,490	p=0,947	p=0,139	p= ---

Bold values (marked correlations) are significant at p < 0,05000

In case of diesel oil contaminated soil the statistical analyses by the use of STATISTICA showed very good correlation between the results of RAMEB-extractions (REH) and the results of dehydrogenase enzyme activity (DEH) and the soil respiration measured in “closed bottle” (RESP CB). A good correlation was also obtained with the HPBCD extracted hydrocarbon content (HEH), the solvent extractable hydrocarbon content (EPH) and the concentration of the oil-degrading cells (MPN).

In case of transformer oil contaminated soil the HPBCD extracted hydrocarbon content (HEH) strongly correlated to the transformer oil-degrading cell concentration (MPN), and also with the soil respiration measured in “closed bottle” (RESP CB).

Our results suggest that the cyclodextrin-extraction can be considered as a chemical model for prediction bioavailability of hydrocarbons in soil. The ability of the RAMEB-, and HPBCD-extraction technique should be further validated by investigation of other organic contaminants over a wide concentration range.

CONCLUSION

Soil biological soil testing methodology was assessed and evaluated in microcosms experiments in order to characterize the biodegradation processes and microbial activity in the soil.

All elements of the applied biological toolbox including simple and cost-efficient microbiological soil testing methods were found to be a reliable indicator of hydrocarbon biodegradation the soil, and can be useful as a pre-implementation methodology to support technology selection and design in case of hydrocarbons contaminated soils.

The aqueous hydroxypropyl beta-cyclodextrin (HPBCD) and randomly methylated beta-cyclodextrin (RAMEB) extraction techniques were also assessed for its applicability to determine of the biodegradable fraction of hydrocarbons in soil. The RAMEB-extract of diesel oil contaminated soil correlated positively with the results of dehydrogenase enzyme activity and the soil respiration measured in “closed bottle” in case of diesel oil contaminated soil. A good correlation was also obtained between the HPBCD extracted hydrocarbon (diesel oil) content (HEH) and the concentration of the diesel oil-degrading cells (MPN).

In case of transformer oil contaminated soil the HPBCD extracted hydrocarbon content (HEH) strongly correlated to the transformer oil-degrading cell concentration (MPN), and also with the soil respiration measured in “closed bottle” (RESP CB).

The results of these experiments suggested that this chemical method, namely cyclodextrin-extraction seems to be a useful tool for prediction of bioavailability of contaminants in soil. Further validation of this chemical model using RAMEB- and HPBCD-extractions is needed with additional poorly available contaminants for estimation of bioavailable fraction of organic contaminants in soil.

REFERENCES

Alef, K. (1995a) Estimation of microbial activities. Dehydrogenase activity, In: Alef, K, & Nannipieri, P. (Eds) Methods in Applied Soil Microbiology and Biochemistry, Academic Press London, UK, pp. 228–231

Alef, K. (1995b) Soil respiration, In: Alef, K, & Nannipieri, P. (Eds) Methods in Applied Soil Microbiology and Biochemistry, Academic Press London, UK, pp. 214–219

Allan, I.J., Semple, K.T., Hare, R. and Reid, B.J. (2006) Prediction of mono- and polycyclic aromatic hydrocarbon degradation in spiked soils using cyclodextrin extraction, *Environmental Pollution,* 144, 562–571

Balba, M.T., Al-Awadhi, N. and Al-Daher, R. (1998) Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation, *Journal of Microbiological Methods*, 32, 155–164

Bardi, L., Mattei, A., Steffan, S. and Marzona, M. (2000) Hydrocarbon degradation by a soil microbial population with ß-cyclodextrin as surfactant to enhance bioavailability, *Enzyme and Microbial Technology*, 27, 709–713

Critto, A., Torresan, S., Semenzin, E., Giove, S., Mesman, M., Schouten, A.J., Rutgers, M. and Marcomini, A. (2007) Development of a site-specific ecological risk assessment for contaminated sites: Part I. A multi-criteria based system for the selection of ecotoxicolgical tests and ecological observations, *Science of the Total Environment,* (379), 16–33

Fava, F., Di Gioia, D., Marchetti, L. (1998) Cyclodextrin effects on the ex-situ bioremediation of a chronically polychlorobiphenyl-contaminated soil, *Biotechnology and Bioengineering*, 58, 345–355

Fava, F. and Ciccotosto, F.V. (2002) Effects of Randomly Methylated- b -Cyclodextrins (RAMEB) on the bioavailability and aerobic biodegradation of polychlorinated biphenyls in three pristine soils spiked with a transformer oil, *Applied Microbiology and Biotechnology,* 58, 393–399

Fava F, Di Gioia D, Marchetti L, Fenyvesi E & Szejtli J (2002) Randomly methylated □-cyclodextrins (RAMEB) enhance the aerobic biodegradation of polychlorinated biphenyls in aged-contaminated soils, *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 44, 417-421

Fava F, Bertin L, Fedi S and Zannoni D (2003) Methyl-ß-cyclodextrin enhanced solubilization and aerobic biodegradation of polychlorinated biphenyls in two aged-contaminated soils. *Biotechnology and Bioengineering*, 81, 384-390

Fenyvesi, É., Gruiz, K., Verstichel, S., De Vilde, B., Leitgib, L., Csabai, K. and Szaniszló, N. (2005) Biodegradation of cyclodextrines in soil, *Chemosphere,* 60, 1001–1008

Frische, T. and Höper, H. (2003) Soil microbiological parameters and luminescent bacteria assays as indicators for in situ bioremediation of TNT-contaminated soils, *Chemosphere*, 3(50), 415–427

Gruiz, K., Fenyvesi, É., Kriston, É., Molnár, M. and Horváth, B. (1996) Potential use of cyclodextrins in soil bioremediation, *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 25(1–3), 233–236

Gruiz, K. (2003) Interactive ecotoxicity tests for contaminated soil, In: ConSoil 2003, Proceedings, CD of the 8th International FZK/TNO Conference on Contaminated Soil, Forschungszentrum Karlsruhe, Wissenschaftliche Berichte, FZKA 6943. (Editor(s).: G.J. Annokkée, F. Arendt and O. Uhlmann) Forschungszentrum Karlsruhe GmbH Publisher, Karlsruhe, pp. 267– 275

Gruiz, K. (2005) Biological tools for the soil ecotoxicity evaluation, In: Innovative Approaches to the Bioremediation of Contaminated Sites –European Summer School Sept 7–11, 2004. Soil Remediation Series No 6., (Editor(s): Fava, F. and Canepa, P.) INCA, Venice, Italy pp. 45–70

Gruiz, K., Molnár, M. and Fenyvesi, É. (2008) Evaluation and verification of soil remediation, In: Environmental Microbiology Research Trends, (Editor(s): Kurladze, G.V.), Nova Science Publishers, Inc. NY, US, pp. 1–57

Ko SO, Schlautman MA & Carraway ER (2000) Cyclodextrin-Enhanced Electrokinetic Removal of Phenanthrene from a Model Clay Soil, *Environmental Science and Technology*, 34(8), 1535–1541

Leitgib, L, Gruiz, K., Molnár, M. and Fenyvesi, É. (2003) Bioremediation of Transformer Oil Contaminated Soil – In: ConSoil 2003, Proceedings, CD of the 8th International FZK/TNO Conference on Contaminated Soil, *Wissenschaftliche Berichte*, FZKA 6943. (Eds.: G.J. Annokkée, F. Arendt and O. Uhlmann) Forschungszentrum Karlsruhe GmbH Publisher, Karlsruhe, pp. 2762–2771

Lorch, H.J., Benckieser, G. and Ottow, J.C.G. (1995) Basic methods for counting microorganisms in soil and water. In: Alef, K. & Nannipieri, P. (Eds) Methods in Applied Soil Microbiology and Biochemistry, Academic Press London, UK, pp. 146–161

Margesin, R., Zimmerbauer, A., and Schinner, F. (2000) Monitoring of bioremediation by soil biological activities, *Chemosphere*, 40, 339–346

McCray, J.E. and Brusseau, M.L. (1998) Cyclodextrin-enhanced in situ flushing of multiple-component immiscible organic liquid contamination at the field scale: mass removal effectiveness, *Environmental Science and Technology*, 32, 1285–1293

McCray, J.E. and Brusseau, M.L. (1999) Cyclodextrin-enhanced in situ flushing of multiple-component immiscible organic liquid contamination at the field scale: analysis of dissolution behavior, *Environmental Science and Technology*, 33 (1), p. 89–95

Molnár, M., Fenyvesi, É., Gruiz, K., Leitgib, L., Balogh, G., Murányi, A. and Szejtli, J. (2002) Effects of RAMEB on Bioremediation of Different Soils Contaminated with Hydrocarbons, *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 44, 447–452

Molnár, M., Leitgib, L., Gruiz, K., Fenyvesi, É., Szejtli, J. and Fava, F. (2005) Enhanced biodegradation of transformer oil in soils with cyclodextrin – from the laboratory to the field, *Biodegradation,* 16, 159–168

Molnár, M., Gruiz, K. and Halász, M. (2007) Integrated methodology including toxicity test-battery to evaluate the bioremediation potential of creosote-contaminated soils in lab-scale experiments, *Periodica Polytechnica*, 51/1, 23–32

Oláh, J., Cserháti, T. and Szejtli, J. (1988) ß-ciklodextrin enhanced biological detoxification of industrial wastewaters, *Water Research*, 22 (11), 1345–1351

Reid, B.J., Semple, K.T. and Jones, K.C. (1998) Prediction of bioavailability of persistent organic pollutants by a novel extraction technique. In: Contaminated Soil'98 (Preprints of the International Conference on Contaminated Soil, Edinburgh, May 17-21, 1998) Thomas Telford Publishing, London, pp. 1207–1208

Reid, B.J., Stokes, J.D., Jones, K.C. and Semple, K.T. (2000) Nonexhaustive cyclodextrin-based extraction technique for the evaluation of PAH bioavailability, *Environmental Science and Technology*, 34, 3174–3179

Sabaté, J. Viňas, M. and Solanas, A:M. (2004) Laboratory-scale bioremediation experiments on hydrocarbon contaminated soils, *International Biodeterioration & Biodegradation*, 54, 19–25

Sheremata, T. W. and Hawari, J. (2000) Cyclodextrins for desorption and solubilization of 2,4,6–Trinitrotoluene and its metabolites from soil, *Environmental Science and Technology*, 34(16), 3462–3468

Szaniszló, N., Fenyvesi, É. And Balla, J. (2005) Structure-stability study of cyclodextrin complexes with selected volatile hydrocarbon contaminants of soils, *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 53(3–4), 241–248

Szécsényi-Nagy, Z., Gruiz, K., Molnár, M. and Balogh, G. (2003) Adaptation of the Microflora to Soil Contaminants, In: ConSoil 2003, Proceedings, CD of the 8th International FZK/TNO Conference on Contaminated Soil. *Wissenschaftliche Berichte*, FZKA 6943. (Eds.: Annokkée, G.J., Arendt, F. and O. Uhlmann, O.) Forschungszentrum Karlsruhe GmbH Publisher, Karlsruhe, pp. 2772–2780