Poster Session C: Remediation Technologies and Concepts

BIOREMEDIATION OF TRANSFORMER OIL CONTAMINATED SOIL

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Introduction

Transformer oil causes frequent soil contamination in Hungary, mainly at transformer stations. Several hundred of transformer oil contaminated sites are on the priority lists of clean-up projects of different industries and the main power-supplier, the Electricity Works. A broad survey showed that transformer oil pollution occurs in both of the saturated and the unsaturated zone of the soil. Most of the contamination at transformer-stations and related industries derives from the PCB free transformer oil which has been used in Hungary for the last 20 years (Product TO 40: Hungarian Standard 153/3-78 Electrical insulation oils, doped transformer oil) and only a few sites had PCB-s measured above the allowable limit concentration (3000 mg/kg soil for Total Petroleum Hydrocarbon; fixed in ministerial decree 10/2000).

Our laboratory experiments proved the biodegradability of the transformer oil in the contaminated soil and indicated significant biodegrading activity in case of six inherited, long-term contaminated sites. This fact let us identify the objectives of a complex technology research: to make the groundwork with this pollutant, to identify the proper remediation technologies, to establish a methodology for the selection of the best possible bioremediation technology, and the technological parameters and the most adequate technology monitoring in case of transformer oil contaminated sites. To demonstrate our results we present the field experiments of bioremediation on three sites contaminated with transformer oil.

To select the best possible remediation technology and technology parameters we assessed the contaminated sites by an integrated methodology and tested the biodegradation and its enhancement in laboratory microcosms. The integrated methodology for testing the contaminated soil shows not only the quality and quantity of the contaminant and the characteristics of the soil, but also the biological status, the scale of adaptation, the biodegradative potential, the mutual and complex interactions between all of the participants: three soil phases, several hundred compounds of the contaminant and the different members of the soil microflora. Also the toxicity and its changes were monitored during the process, which maybe proportional with the bioavailability of the contaminant and may call our attention on the chemically not measured toxic compounds and the chemical time bomb phenomena (Gruiz et al, 2001) at the site and in the contaminated soil. The risk of the contaminated soil can be directly characterised by a proper ecotoxicity test-battery.

The microcosm tests reflect the dynamics of the system and the possibility of enhancing the general microbial activity and the biodegradation of the contaminant in the soil. Also technological parameters

have been determined in similar experiments, like maximal aeration rate, nutrient supply or the necessity of bioavailability enhancing agents or a microbial inoculum.

The intensity of hydrocarbon biodegradation is influenced by many factors such as quality, quantity and bioavailability of the contaminant, nutrient supply and availability, soil properties, like pH, temperature, water content and quality, quantity and biodegradative activity of the soil microflora.

The bioavailability of contaminants may also be a problem in the bioremediation of soil contaminated with hydrocarbons. Cyclodextrins enhance desorption of apolar contaminants from the solid surface and increase their concentration in the aqueous phase of the soil, in the biofilms, improve the availability of these compounds to the hydrocarbon-degrading microbes (Fenyvesi, 2002).

Objectives

Two case studies are introduced in this presentation to argue for the bioremediation of transformer oil contaminated soil, and to demonstrate the complex approach used during the process of technology selection (NRDP, 2001-2004),

In each case significant biological activity was measured in the soil. The microflora was able to degrade the transformer oil contaminant after its enhancement by aeration, nutrients and additives, even in the case of a freshly contaminated soil or a bad quality industrial filling.

Cases differ from each other in terms of soil type, contaminated soil phases, and the used additives. The technology was followed by an integrated methodology (Biotechnology, 2000–2003, Poster presented by Monika Molnar, Gruiz et al, 2001): a special battery of physico-chemical, biological and ecotoxicity test-methods, which has been developed for the monitoring of bioremediation.

The positive result of the laboratory biodegradation tests indicates the selection of technologies based on the natural microbial activity of the soil. For the enhancement of the natural biodegradation an environment optimal for the biodegrading microflora is to be ensured by aeration, nutrient supply, bioavailability enhancing agent, microbial inoculum (bioaugmentation) and other additives.

In **Case I**. a complex methodology: *in situ* bioventing of the unsaturated zone was combined with the ex situ treatment of the water and temporary *in situ* flushing of both the saturated and unsaturated zones, in **Case II.** an agrotechnical *in situ* bioremediation of the upper 1,0–1,5 meter of the soil was applied.

Experimental

In this chapter we introduce the organisation of the framework projects, characterise the contaminated sites and soils and describe the applied materials and methods.

The field experiments were parts of Hungarian and International Research Projects (NATO, 1999–2003 and NRDP, 2001–2004, Biotechnology 2000–2003). The theoretical background of soil remediation, the microbiology and technology research was developed by the Budapest University of Technology and Economics (BUTE), the physico-chemical soil characterisation by the Research Institute for Soil Science and Agricultural Chemistry of Hungarian Academy of Sciences, the chemical analysis and cyclodextrin-research by the Cyclolab Research and Development Laboratory. For the implementation of the field experiment small size Hungarian enterprises were responsible.

The field experiments were planned and implemented after a long preparatory work that included: assessment of the site, identification of the contaminant, the contaminant source(s) and the site specific characteristics of the environment, creation of a transport and an exposure model, creating the target value, testing natural attenuation and its enhancement, selection of all possible technologies, processing technology experiments, determining the optimal technology parameters, comparing all possible technologies on the basis of a cost efficient assessment and the selection of the best possible technology. In this paper the implementation of the selected technology and its monitoring is discussed. The selected technologies are all based on the natural biodegradative activity of the soil, the monitoring applies an integrated methodology using physico-chemical analyses, biological and ecotoxicity testing.

Site I. A long-term contamination derived from a leaking and regularly refilled spare-transformer on a transformer station. The oil from the point source has already reached the groundwater when it was discovered. The transformer could not be removed from its place, so an *in situ* treatment was applied without using the surface beyond the contaminated subsurface spot. Both the saturated and the unsaturated zones were treated and a complex technology was applied: an *in situ* bioventilation of the unsaturated zone, ex situ water treatment after pumping ground water to the surface and *in situ* flushing of both the saturated and unsaturated zones.

Contaminant of Site I.: PCB free transformer oil TO 40

Contaminated phases: unsaturated and saturated zones of the soil, ground water

Water table: 2.6 metres, reached by the contaminant

Volume in the field experiment: 90 m^3 (150 tons) Type of the soil: sandy soil, with a permeability coefficient of k = 1,03 m/s

Average concentration of the contaminant: 25 000 mg/kg soil

Average concentration of the contaminant in the ground water: 0.99 mg/litre.

Toxicity of the soil: the soil was characterised as "slightly toxic" in the upper 10-20 cm, but "very toxic" in 1 m depth. Test organisms of three trophic level were used: *Vibrio fischeri, Sinapis alba* and *Folsomia candida*.

Characterisation of the initial soil microflora: concentration of the aerobic heterotrophic cells: $2*10^7$ CFU/g soil; of the transformer oil degrading cells: $2–3*10^4$ cell/g soil.

Result of the microcosm tests: cell concentrations could be increased in microcosm tests by aeration, nutrients and by enhancing bioavailability. The respiration and the biodegradation rate increased meaningfully in the experiments.

Technologies applied in the Site I. field experiment:

Total duration of the field experiment: 8 months, included 3 months winter break

Periods and phases of the treatment: 1. Continuous bioventilation: 1a.) 2,5 month bioventilation, until stabilisation of the stationary phase; 1b.) 2,5 month period for three-times addition of nutrients (N, P, K) and RAMEB to the unsaturated zone. 2. Application of a pump and treat technology for the contaminated water: 2a.) 2,5 month pump and treat until stabilisation of the stationary

phase (constant water quantity and quality); 2b.) 2,5 month period for three-times addition of nutrients (N, P, K) and RAMEB through the unsaturated zone. 3. A mild flushing with the treated groundwater: 3a.) 2,5 months no flushing, only irrigation to recover soil humidity; 3b.) 2,5 months constant mild flushing and occasional flooding together with nutrient and RAMEB supply; 3c.) Continuing mild flushing and occasional flooding.

Bioventilation of the unsaturated zone: a slow airflow is produced by a low performance ventilator through aeration wells equipped with perforated casing. 2 active (sucked) and 2 passive wells (inlet of atmospheric air) have been arranged on the right and left side of the transformer. One of the active wells is a simple air-exhausting well, the other is a combined one for exhausting soil-air and pumping out ground water. The upper part (close to the surface) of the passive wells have been entwined, forming a larger trench for air inlet and flooding by water. The frequency of air exchange cycles in the ventilated soil volume is minimum 1 air exchange/h, that means 20-30 m^3/h air flow. The type of the ventilator is Siemens ELMO 2BH7-3G, its maximal air delivery is 50 m^3/h, power: 0,55 kW.

Pump and treat technology the water pump is a Grundfos SP5 type plunger pump, placed into the combined well. The amount of the pumped water is 5-6 m^3/hour. The treatment of the water is a three step treatment: after phase separation, a thieving on sand filter and following adsorption on activated carbon is applied. The treated water is reserved in a closed tank (25 m^3) and is used for the irrigation and the flushing of the treated soil volume and for the injection and infiltration of the additives. (0,5–0,6 m^3/h).

Flushing: mild flushing combined with a temporary complete loading of the contaminated soil volume.

Nutrients supply: N, P and K containing fertilizers were applied three times 60 kg artificial fertiliser.

Enhancement of bioavalability: randomly methylated beta cyclodextrin (RAMEB) was applied to improve the desorption of the contaminants strongly adsorbed on the surface of the soil particles and increase the contaminant concentration in the aqueous biofilms where the microbes work. Three times 10 kg aqueous RAMEB solution containing 50 % RAMEB (Cawasol W7 MTL, Wacker Chemie, Munich) was added together with the nutrients dissolved in 1 or 2 m^3 water. The moisture, nutrients and additives have been supplied through the perforated casing of the active and passive wells.

Integrated methodology for the initial assessment: a.) measuring of the physico-chemical soil characteristics (soil type, infiltration coefficient, nutrient content); b.) chemical analysis of the contaminant by gravimetry and gas-chromatography; c.) characterisation of the soil microflora: concentration of aerob heterotrophic and transformer oil degrading cells; d.) microcosm testing: measuring the effect of aeration, nutrients and RAMEB on the cell concentrations, respiration and on the decrease in the amount of the contaminant; e.) ecotoxicity testing of the whole soil by direct contact tests with the testorganisms of three trophic levels: bacteria *(Vibrio fischeri*) plant *(Sinapis alba)* and animal *(Folsomia candida).*

Integrated methodology for the technology monitoring: as the treated site is physically unavailable from the surface, the technology monitoring was designed on the basis of analysing the exhausted soil gas and the pumped and treated ground water. The CO_2 and O_2 content of the

soil gas is proportional with the microbiological activity, the oil content of the ground water shows directly the biodegradation. a.) physico-chemical measurements: amount and humidity of the exhausted soil-air, amount of the pumped ground water, b.) chemical analysis: extract content of the ground water by gravimetry and gas-chromatography with flame ionization detector, CO_2 and O_2 content of the exhausted soil air by gas sensor (type Oldham MX 21).

Integrated testing of the soil: soil has not been sampled during the remediation process, but the soil analysis was carried out only before and after the application of the technology. Complete physico-chemical, biological and ecotoxicity testing was done: concentration of the contaminant measured by gravimetry and gas chromatography, cell concentrations and ecotoxicity.

Site II. Chronic and repeated contamination was identified on the open storage-site of a transformer station of a cement factory. Only the unsaturated zone of the soil has been contaminated at a depth of 1–1,5 meters. The subsurface water has not been endangered, because of the low water table and the existence of an isolating loamy layer in a depth of about 3 metres. The soil is a heterogeneous refilling with unmapped hard loamy clods under the surface. The selected biotechnology is an *in situ* treatment, applying agrotechnologies (tilling, irrigation) and injection of amendments and bacterial inoculum.

Contaminant of Site II: PCB free transformer oil TO 40 mixed up with a few heating-oil

Contaminated phases: unsaturated zone of the soil

Water table: is low, under 3 metres

Contaminated soil volume: 100 m^2

Type of the soil: heterogeneous refilling

Average concentration of the contaminant: 20 000 mg/kg soil

Minimal and maximal concentrations of the contaminant: 5 000 – 78 000 mg/kg soil

Infiltration depth: a maximum of 1.5 meter, but the highest concentration was found in the upper 0,5 meter.

Toxicity: the soil was characterised as "very toxic" from the results of three bacterial inhibition tests: *Azotobacter agile* dehydrogenase-activity; *Pseudomonas fluorescens* growth and *Vibrio fischeri* bioluminescence.

Characterisation of the initial state of the soil microflora: concentration of the aerobic heterotrophic cells: $1–2*10^6$ CFU/g soil, of the oil degrading cells: $2–3*10^3$ cell/g soil

Result of the microcosm tests: only a little increase in the cell concentrations was observed by the enhancement.

Bioaugmentation: the artificially prepared site-specific inoculum together with a nutrient supply resulted an enhanced biodegradation. The self made, site-specific inoculum, which consists of 15 bacteria isolated and selected from the soil of the site was propagated in the laboratory. After inoculation the cell concentration of the aerobic heterotrophic cells increased to a value of $1–2*10^8$ CFU/g soil and the transformer oil degrading cell concentration to $2–3*10^5$cell/g soil, the respiration and the biodegradation rate increased meaningfully in laboratory experiments.

Technology applied in the in situ field experiment in Site II.

Total duration of the field experiment: 18 month

Periods and phases of the treatment: 1. Application of agrotechnologies: tilling, irrigation, nutrient supply and bioaugmentation to maintain optimal environment for biodegradation: 1a.) 1 month preparatory works; 1b.) 3 months: inoculation monthly with the self-made site-specific bacterial inoculum; 1c.) 3 months treatment with agrotechnologies; 1d.) 4 months winter break; 1e.) 2 months soil treatment; 2. Injection of the microaerophyl inoculum into the deeper layers targeted the loamy clods: 2a.) 3 months inoculation monthly with microaerophyl bacteria 2b.) 2 more months soil treatment by agrotechnologies.

The treatment bed: a natural isolation ensures the safety: there is no risk for transport of the contaminant into subsurface waters, so the soil was let in place. To improve aerobic conditions a manual digging was applied every two weeks. Natural precipitation was complemented with irrigation. Artificial fertilisers of commercial origin supplemented N and P.

Bioaugmentation in two steps: 1. Increasing quantitatively the cell concentration of the indigenous microflora by bacteria isolated from the site and grown in the laboratory. 2. Qualitative modification of the natural microflora by a commercially available microaerophyl bacterial product.

Integrated methodology for the initial assessment: a.) measuring of the physico-chemical soil characteristics (soil type, humus content, humidity, nutrient content); b.) chemical analysis of the contaminant by gas-chromatography; c.) characterisation of the soil microflora by measuring of cell concentrations, d.) microcosm testing of the effect of aeration, additives and inoculants on the cell concentrations, respiration and elimination of the contaminant; e.) ecotoxicity testing of the whole soil by direct contact tests with three testbacteria *(Vibrio fischeri, Azotobacter agile, Pseudomonas fluorescens).*

Integrated methodology of the technology monitoring: a.) physico-chemical; b.)chemical; c.) microbiological and d.) ecotoxicity testing of the whole soil by three bacteria.

Test methods: part of the used methods are equivalent with standard methods (measuring of the extractable petroleum hydrocarbon by gas-chromatography with flame ionization detector HS 21470, 1994, Hungarian Standard; Microbiology examination HS 21470/77, 1988, Hungarian Standard, Soil Quality – Effects of soil pollutants on Collembola (*Folsomia candida*) ISO/DIS 11267, 1994, Draft International Standard), some others are based on standard method but applied after modification (*Azotobacter agile* dehydrogenase enzyme activity test HS 21978/30, 1988, Hungarian Standard, *Vibrio fischeri* bioluminescence test DIN 38412, 1991, German Standard; *Sinapis alba* root and shoot elongation test HS 21976/17, 1991, Hungarian Standard) but some of the applied methods are developed by our research group, like, transformer oil degrading cell number, *Pseudomonas fluorescens* growth inhibition HS 21470/77, 1988.

Results and discussion

Laboratory experiments proved that the transformer oil is biodegradable in the humic soil: close to 40 % is removed in four weeks. RAMEB increased the mobilisation of transformer oil immediately after adding to the soil. RAMEB shortens the lag-phase of the bioremediation (Figure 3.)

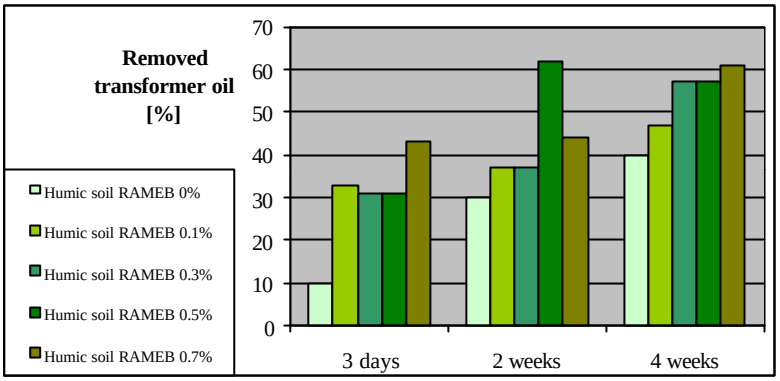

Figure 3.: Bioremediation of transformer-oil contaminated soil

Site I. Field experiment with a combined technology

Three technologies were applied at the same time for Site I.: treatment of the unsaturated zone by bioventilation, intensification of the biodegradation with additives, like nutrients and RAMEB; pumping and ex situ treatment of the ground water and temporary *in situ* flushing for the saturated and unsaturated soil zones with part of the water treated on the surface.

The treated soil was unavailable for sampling, so the technology monitoring was solved by the soil air and the ground water sampling and analysis. The results are shown on Figure 9.

The effect of the first RAMEB + nutrient addition is remarkable: the increase of the CO_2 content and decrease of the O_2 content shows the suddenly increased activity of the microflora in the soil. The second amendment brought a little step and the third no increase any more in the CO_2 production. We supposed that the biodegradation of the transformer oil has already happened, so no substrate (energy source) was available any more.

Water samples have been regularly analysed for dissolved hydrocarbon content. The results can be seen in Figure 10. The initial oil content of the ground water was 0.99 mg/l, it decreased to 0.8 mg/l in the initial stationer state, after 40 days of aeration. On the effect of the first RAMEB + nutrient treatment it decreased to 0.6, after the second treatment to a value of 0.4 mg/l.

The hydrocarbon content of the soil under the transformer decreased from 25 000 mg/kg to 2 000 mg/kg in the depth of 10-30 cm and to 900 mg/kg in the 80-90 cm, as a result of the *in situ* bioremediation. The soil close to the surface was not available from the surface, and was also excluded from the mainstream of the technology, which applied horizontal air flow and flushing from a subsurface ditch.

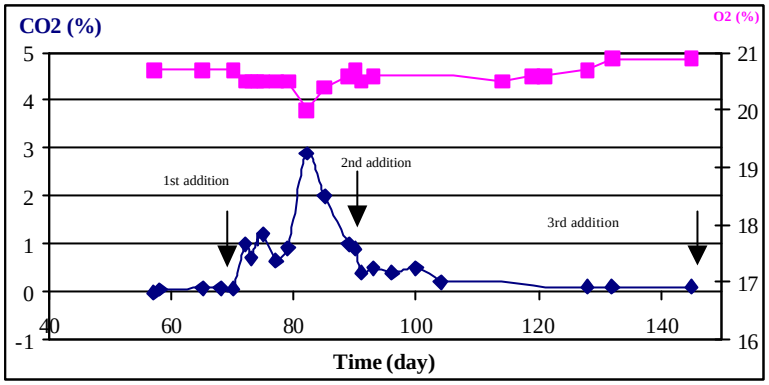

Figure 4. CO_2 and O_2 content of the exhausted soil air during the *in situ* treatment

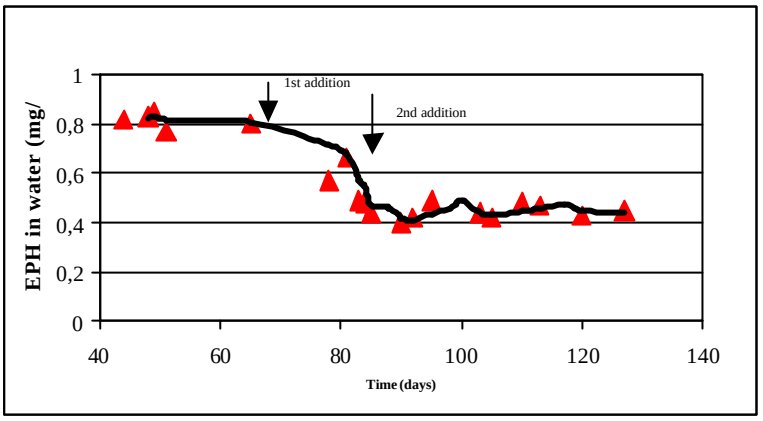

Figure 5. EPH content (mg/litre) of the ground water during the *in situ* treatment

Table 1. Ecotoxicity of the transformer oil contaminated soil before and after the *in situ* treatment

Depth of contaminated soil	10-30 cm		80-90 cm	
Date of testing	*Before*	*After*	*Before*	*After*
Vibrio fischeri ∑Cu20 [mg Cu/kg sample]	320	230	450	<80
Sinapis alba ED_{20} [g]	3	>5	2	>5
Sinapis alba ED_{50} [g]	4	>5	2	>5
Folsomia candida ED_{20} [g]	10	>20	4	>20
Folsomia candida ED_{50} [g]	12	>20	5	>20

Results of *Sinapis alba* root and shoot elongation test, *Vibrio fischeri* bioluminescence test and *Folsomia candida* mortality test are shown in Table 1. The plant and the animal testorganisms show a decrease in the toxicity, with an acceptable end-value. The *Vibrio fischeri* indicates a mild toxicity in the upper 10-30 cm layer of the contaminated soil, but no toxicity in the deeper one, what agrees with the oil content.

Site II.: results of the field experiments

The laboratory experiments showed slow biodegradation even after enhancement with nutrients and aeration. That leads us to conclude that a low cell concentration was in the industrial soil. A site-specific inoculum, which prepared from 15 bacteria isolated from the site and grown in laboratory fermenters, increased biodegradation rate and cell number in laboratory experiments (table 2.).

Table 2. Changes in the cell counts after inoculation and adaptation for 1 month: laboratory experiment

Experiments	**Aerob heterotrophyc CFU/g soil**	**Oil degrading Cell/g soil**
Original soil without adaptation	$1.8* 10^6$	$3.5* 10^3$
Original soil after adaptation and enhancement	$4.0*10^6$	$5.5* 10^3$
Inoculation with self made site specific bacteria "A"	$7.4 * 10^8$	$1.9 * 10^6$
Inoculation with self made site specific bacteria "B"	$1.2 * 10^9$	$4.6 * 10^5$
Self made inoculum of indigenous and alien bacteria	$4.5 * 10^8$	$4.6 * 10^5$

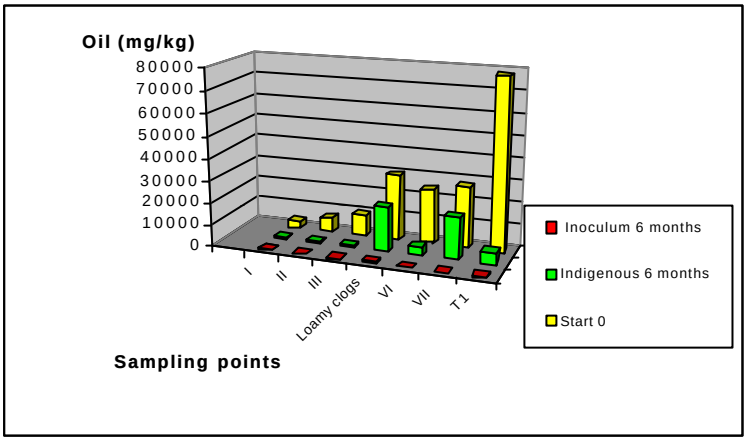

Figure 6. Oil content in the soil after the treatment

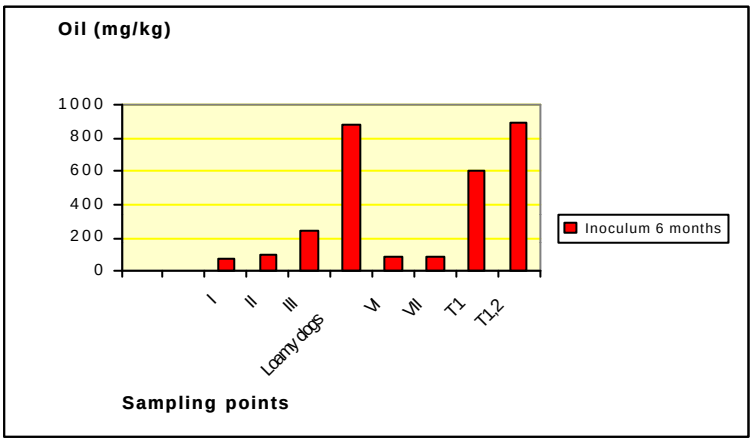

Figure 7. Oil content in the soil treated by microaerophylic bacterial inoculum

We applied the same supplements and inoculum for the field experiments (green columns on Figure 6.). After 6 month treatment contamination has been reduced in most part of the site onto a value of 3–5 000 mg/kg, but in some spots the remaining concentration was 15–20 000 mg/kg.

After detailed exploration subsurface loamy clods were found in heterogeneous distribution with high oil content and anoxic environment. A commercially available microaerophylic bacterial inoculum was applied first in laboratory experiments, then at the field. 6 more months brought the aimed result, shown on Figure 6. and 7 (red columns).

Conclusion

Field experiments were established to study the application of *in situ* bioremediation for transformer oil and reproduce good laboratory results. The bioremediation based on the natural microbial activity was enhanced in every case by aeration and nutrient supply, in one case by two different inoculum applied consecutively, in the other case with RAMEB, an environment-friendly mobilising agent of natural origin.

The assessment of the contaminated site and the technology monitoring applied an integrated methodology with physico-chemical, biological and ecotoxicological testing. The case studies showed some examples, to demonstrate how this integrated methodology could be used and what additional information could they give for the selection, monitoring and evaluation of the technology.

Site I. represents a typical situation, when transformer oil contamination exists in more soil phases: in the ground water, in the saturated and unsaturated soil zones. A combined technology of *in situ* bioventilation*, in situ* flushing and ex situ treatment of the pumped ground water with the addition of RAMEB and nutrients resulted in a fast reduction of the transformer oil content in the ground water and in the soil. Technology monitoring was based on soil gas and ground water analyses. During the remediation, the oil content of the ground water decreased from 0.99 mg/l to 0.4 mg/l. After finishing the treatment the soil was completely analysed: its oil content decreased from 25 000 to 900 mg/kg.

Site II. was an industrial site with bad quality soil, which showed low cell concentration in spite of chronic and repeated pollution. Biodegradation was enhanced in two steps: aeration, nutrient supply and addition of a site specific bacterial inoculum of externally grown indigenous microflora. The biodegradation rate increased, but the result was still not acceptable, mainly due to anoxic loamy clods. A second step of augmentation with a microaerophyl bacterial soil inoculum brought the desired result both in terms of reduced oil content and toxicity.

Acknowledgement:

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NRDP 2001-2004 Development of complex and efficient technologies for remediation of contaminated soils

OMFB-Biotechnology 2000-2003 Methods to monitor bioremediation processes (BIO-00066/2000)