INTERACTIVE ECOTOXICITY TESTS FOR CONTAMINATED SOIL

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Soil is a complex system, with three phases: gaseous, liquid and solid, however the biota and the habitat of the soil microflora, the special micro-surfaces and bio-films having special role and characteristics can be considered as a fourth phase.

The contaminants of the soils may also be complex mixtures of chemicals, and the interactions between soil phases, including the biota and the components of the contaminant results in endless combinations. Additionally there is no equilibrium state in the soil after a contamination event, but a continuously changing environment that will be responsible for the actual effects of the pollutants.

The results of sampling and chemical analysis reflects certain co-ordinates in time and space, even if the sampling fulfils the requirement to represent the whole site. The results of the chemical analyses seldom correlate with the actual effects and risks of the contaminant mixture at a certain site. To get a realistic view about the risk of the soil pollutant an integrated approach is needed: complementary biological (ecotoxicity, mutagenicity, teratogenicity, food chain, etc.) testing is necessary to the chemical analysis. The physico-chemical (C) and biological (B) results should be evaluated together. The consistency or the rate and type of inconsistency between C and B gives information on the biological availability of the contaminant, the interaction between the single contaminants (synergism, antagonism) and calls the attention on chemically not measured or not measurable but existing dangerous components through their adverse effects (Gruiz at al, 1999).

Many type of bioassays are known, some of them have already been standardised, some others are under development (Calow, 1993; Landis and Ming-Ho Yu, 1999). The selection and the application of the proper test-method, including sample pre-treatment, test-organism and test-method depends on the aim of the assessment, which can be: site assessment, toxicity mapping, monitoring, risk assessment, risk characterisation, creation of environmental quality criteria or target quality criteria, direct decision making, remediation technology-monitoring, etc. (Gruiz, et al, 1998; 2000). If the aim of the testing is e.g. to estimate the risk of the soil-contaminant for the ground water (many of the standardised tests aims this) a water-, an acid- (e.g. carbonic acid) or a salt-extract of the soil (modelling the concrete leaching procedure in the soil) and water-living organisms, like algae, *Daphnia* or fish are used for ecotoxicity testing. If the plant uptake of metals is to be modelled organic acids or acetate-buffers are often used, which have similar extracting behaviour than the acidic root fluids.

The soil itself as an individual element of the environment and living habitat of hundreds of organisms has been less in the centre of interest as compared to water. To characterise the actual risk of the contaminant on *soil ecosystem*, organisms representative to this soil ecosystem should be used. An other important rule is: no extract but whole soil (the same with sediments) should be tested, because the partition of the contaminant in the phases of the soil (sediment) is not in equilibrium and depends on the effect of the biota. The selected test-method should ensure the **direct contact** between the soil and the test-organism, so that the interactions between the soil and the test organism and the contaminant and the test-organism can be realised during the testing, to model the real situation in the soil (interactive tests).

In the laboratory single species are often used for the characterisation of environmental samples in simple bioassays. Test organisms for soil testing (bacteria, plants, protozoa, worms, insects, spiders, rodents, etc.) and endpoints (survival, growth, immobilisation, respiration, enzyme activities, reproducibility, etc.) are of wide selection. For characterisation and mapping of contaminated sites also the members of the indigenous (micro)flora and fauna or their biochemical and DNA markers can be used as indicators. According to the duration of the tests they are divided into two groups: acute (short term) and chronic (long term) test. Laboratory testing of contaminated environment may go not only with single species but also with multispecies test-systems, like microcosms, which better represent the real environment: a complex community is living in them, they have their own history and evolution. Of course it cannot faithfully reproduce the entire ecosystem, but secondary effects, like fate and partition of the contaminant, bioaccumulation, site specific biodegradation and the complex interactions can be studied with their help. The two main dilemma are the size and the heterogeneity of the test system: to model the real environment a large size and heterogenic system (like the nature) is needed, but to

create a method of good statistics requires the opposite. A suitable statistical evaluation (graphical interpolation, probit, logit, principal component analysis, nonmetric clustering, etc.) is also needed.

We would like to emphasise that a correct and realistic characterisation of a polluted site or contaminated soil is only possible by using an integrated methodology: physico-chemical, biological and ecotoxicity results should complement each other (Gruiz at al, 1998a,b). Integrated test-batteries have been developed for the complex soil-characterisation of different purposes, like assessment of risk, characterisation of contaminated soil, monitoring of remediation included bioremediation, classification and reuse of treated soil, etc. (Gruiz, 2002). Integrated environmental assessment and monitoring, which evaluates physico-chemical and biomonitoring data together, helps to follow the changes and to identify the acceptable limit of changes in soil characteristics (soil activities, diversity, indicator species, etc.).

The present paper introduces some of the methodological developments and applications of the research group of BUTE Environmental Microbiology. These specific techniques are used for the characterisation of contaminated sites and soils, of the fate, nature, behaviour, partition, bioavailability, biodegradability and bioaccumulation of the contaminant, for planning and monitoring of bioremediation, classification of contaminated or treated soils and for site specific risk assessment. The methods developed in our laboratory belong to three main groups of tests, measuring: 1. the response of single species in bioassays, 2. the population, the activities or any characteristics of the soil and 3. the response of whole soil in microcosms.

1. Laboratory bioassays with single species test-organisms

These methods are all direct contact tests, where whole soil could interact with the test-organism and use well defined, artificially cultivated test-organisms of the same growth phase or a synchronised population.

1a. Bacterial bioassays are easy, fast, well reproducible tests, by an organism, which represents an important part of the soil ecosystem. Most of the test-microbes were isolated and selected by us, some of them derive from culture collections. (Horváth et al, 1996)

Azotobacter chroococcum and Bacillus subtilis growth inhibition by solid soil or sediment. Soil is stabilised with agar-agar gel (without nutrients) and diskettes are cut out from this gel, which are placed on the surface of a relatively high density bacterial culture grown in an agar medium. The inhibition zone around the soil diskette is measured in mm. The inhibition is compared to the concentrations of the identified or supposed contaminating substances (mixed into soil). This fast and simple testing method is suitable for screening of a great number of soil and sediment samples and for toxicity mapping of sites of great extension.

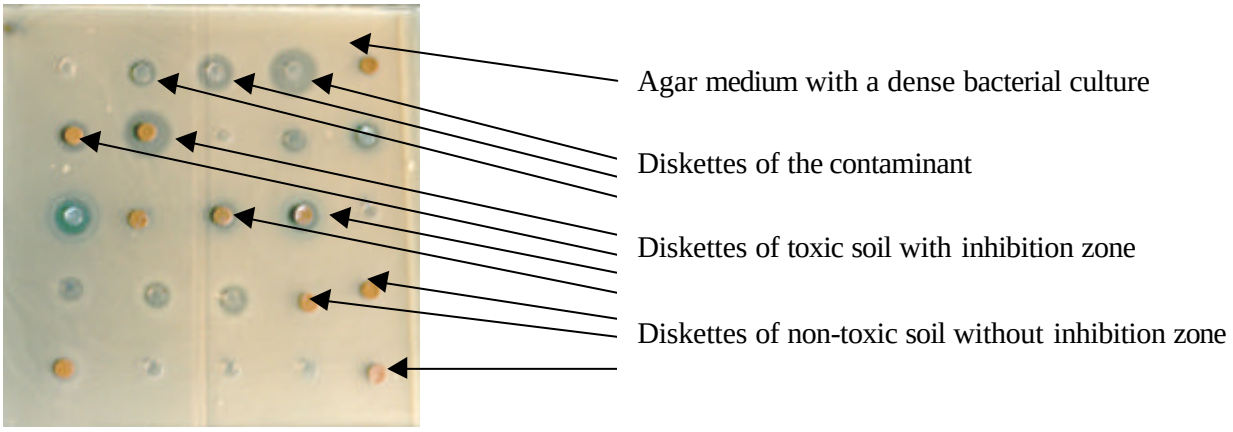

Figure 1. Soil diskettes on the surface of the agar medium with the culture of the test-bacterium

***Vibrio fischeri* bioluminenscence** is measured in soil suspension. This bacteria is not a soil living one, but similar bacteria are members of the soil microflora. It is a well known, standardised test-organism of marine origin, easily grown in laboratory. On the adverse effect of the contaminant a decrease in the intensity of the luminescence can be measured. To ensure the direct contact between soil and bacteria, a soil suspension is added to the test-organism containing media (modification of

DIN 38412, 1991). The endpoints of the assay is ED_{20} or ED_{50}, giving the mass of soil (g) resulting in 20% or 50% decrease in the luminescence intensity, what is measured by a simple luminometer. This primary result can be expressed in Cu-equivalent, measuring a Cu calibration curve under the same conditions as the testing itself. After calibration, the results are given in EC_{20} or EC_{50} (mg Cu in kg soil). Use of this test is very general in our practice for soil characterisation, for site and technology monitoring.

Bacterial dehydrogenase inhibition test runs in growing dilution of the suspension of the contaminated soil. Most often *Azotobacter agile* is used, but other characteristic soil bacteria can also be used, e.g. *Pseudomonas fluorescens*, *Bacillus subtilis* or *Azotobacter chroocuccum*, etc. An alternative electron acceptor, the TTC (2,3,5-triphenyl-tetrazolium-chlorid) is added to the test-medium. If the respiration of the test-organism is not inhibited, a pink colour appears, colour intensity is proportional with the respiration rate. Semi-quantitative result can be obtained by visual evaluation, quantitative result by measuring the colour-intensity as primary endpoint by a simple spectrophotometer, after solvent extraction of the TPF (1,3,5-triphenyl-formasane). ED_{20} and ED_{50} are the endpoints of the test method, giving the mass of the soil resulting in 20 or 50% inhibition in the respiration of the test-organism. The tested soil should be sterile. The result can be expressed as an equivalent of copper or any other toxic substance with the help of a calibration curve. Dehydrogenase inhibition test is used for general testing of contaminated soil and sediment during assessment and remediation.

1b. Direct contact plant tests are interestingly less popular for testing soils as an individual habitat, by the reason that some of the plants are not sensitive enough, some others are too sensitive and many uncertainties are to be met during testing and evaluation. Plants are used mainly for the testing of the extracts of dangerous wastes: generally germination or root elongation are tested, to investigae the risk of waste disposal onto land. Plant tests have increasing importance in the assessment of contaminated land and soils, their result play an important role in risk assessment and in the creation of quality criteria. Their response, as representatives of one of the most important trophic level (producers) in soil, is crucial. If we have to calculate the predicted no effect concentration which do not effect soil ecosystem, we have to use test-organisms of minimum three trophic levels, included plants and extrapolate from the results of the single species to the whole ecosystem. Also bioaccumulation and food chain effects are based on plant behaviour, so plant test have growing importance in ecological and human risk assessment, so that it plays important role in our developments.

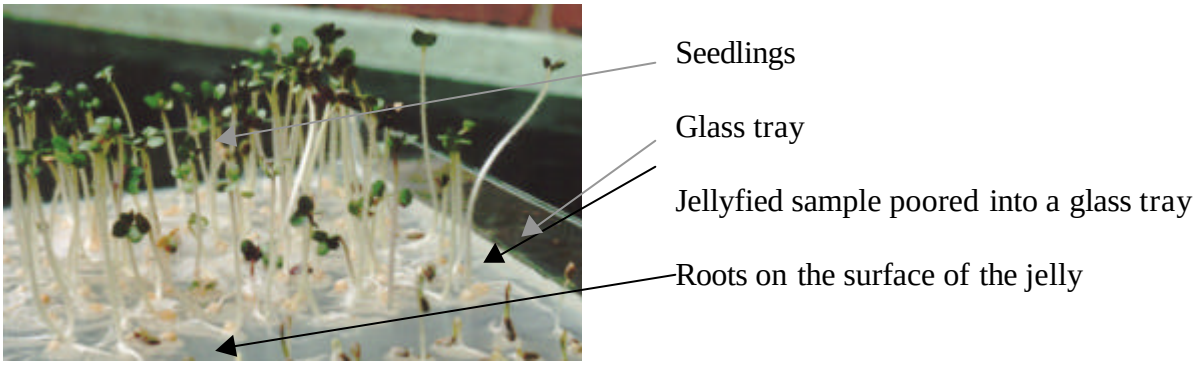

Figure 2. Seedlings on the surface of the jellyfied soil suspension

A large number of plants were examined in our laboratory for testing contaminated soil before, during and after remediation by growth inhibition. White mustard *(Sinapis alba)*, pea (*Pisum sativum*), golden cress (*Lepidum sativum),* radish *(Raphy nus sativus),* wheat (*Triticum sativum*), oat *(Avena sativa)* and maize *(Zea mays)* were used as testplants, they were seeded directly into the soil to ensure the interaction between plant root and soil. Plant growth in acute tests is measured by root and shoot elongation. To measure root lengths is sometimes complicated or even impossible because roots tightly grow into the soil. Two solutions were found to avoid that problem: stabilising soil by agar-agar gel (perfect contact but growth only on the surface) or placing a thin layer of filter paper between soil and plant. Shoot elongation associates better with the contaminant concentration and correlates better with other ecotoxicity test results, than root, because the response of root in soil is often an abnormal elongation (but thinner in morphology) to avoid contaminated soil surface. In chronic plant tests the biomass is also measured and characterised.

Most of the tested plants showed moderate sensitivity in growth tests for different contaminants, pea showed high sensitivity for metals. Test method: a dilution series is prepared from the tested soil with

sand or standard (e.g. OECD) soil. Evaluation: ED20 and ED50 is determined, which gives the mass of soil which causes 20 or 50% growth inhibition. If no curve can be established from the measured result of the dilutions (e.g. no inhibition in the diluted soil) the inhibition can be expressed in the % of the uncontaminated control soil.

Bioaccumulation in plants and through the food chain are of high risk in certain contaminants and soil types. For toxic metal bioaccumulation six test-plants were used: garden-sorrel *(Rumex scutatus*), onion *(Allium cepa),* chieve *(Allium schoenoprasum*), carrot (*Daucus carota*), parsley (*Petroselinum officinale*) and horseradish *(Armoratia rusticana*). Growth and contaminant content in the root and shoot tissue is measured. Results of bioaccumulation tests can be used for risk characterisation, e.g. for secondary poisoning, or for measuring the mobility of contaminants and their availability for plants.

1c. Tests using soil living animals apply generally a direct contact between soil and test-organism. The existing and standardised *Folsomia candida* (Collembola: a springtail) and *Eisenia foetida* (earthworm) are the most popular test-organisms. We modified the methods of Ripert and Kula (1996). The necessary duration of these animal tests are too long, more than two weeks, even in case of acute toxicity testing. To keep the advantage of the animal cells, but shorten test duration we developed protozoon test for the testing of soil suspensions. Protozoa tests need shorter time, but the counting of the living cells is sometimes too difficult.

2. Testing the characteristics of soil ecosystem

Response of the soil's own ecosystem on the adverse effects of pollutants is more realistic, but the evaluation of the damage (toxic effect) is generally not possible on the basis of the measured absolute values of the endpoints like cell concentration, respiration, enzyme activities, diversity of bacteria, plants or animals, etc., because healthy environment is not well characterised and is influenced by season, climate and other environmental parameters. To avoid the problem we have some useful strategies according to the aim of the testing:

2a. The ecosystem of the soil reacts immediately upon any outer effects, of any changes of the environmental parameters, including the foreign substances. The flexibility, the adaptive behaviour of the soil biota and the regulating mechanisms in the soils are generally well developed, but depends on the state, age and type of the soil and also are influenced by seasonal and climate characteristics. Adequate statistical evaluation makes possible the differentiation between the healthy average and the affected soil. In our research soil microflora is investigated quantitatively or qualitatively. Concentrations of aerob heterotrophic bacteria, filamentous bacteria, fungal species, anaerobic or facultative anaerobic colony forming cells, etc., their activities, like respiration, dehydrogenase activity, nitrification, ammonification, etc., the population diversity and physiology shows characteristic changes on the adverse effect of contaminants. A method e.g. used by Dobler at al. (2001) is able to clearly discriminate the polluted soil from the pristine ones on the basis of the substrate utilisation pattern of the soil microflora, determined with the commercially available Biolog system.

2b. Selective biological, biochemical and genetic markers are useful to indicate the presence and/or to characterise the effect of certain contaminants. Adaptation of the soil ecosystem to the contaminant can be followed or proved by indicating the existence of resistant strains of soil microbes or by those, which are able to degrade the contaminants/xenobiotica. The cell concentration of the pollutant-degrading microbes in a soil can be measured by any growth- or respiration-tests applying the contaminant as the only carbon source in the test system containing whole soil. We developed a relatively simple test for measuring the concentration of hydrocarbon (or any organic xenobiotica) degrading cells in soil. A dilution series is prepared from the soil in form of a suspension in a mineral medium. Then equal amount of the contaminant (if the contaminant is not identified it can be extracted from the polluted soil) and an alternative electron acceptor (generally INT = (2-(p-iodophenyl)-3-nitrophenyl)-5-phenyltetrazolium-chlorid)is added in all tubes of the series. After a few days or weeks incubation the "most probable cell number" is determined by statistical evaluation. Plating or other cell counting methods are also suitable. Resistance of microbes is also a useful property for indicating pollution and adaptation by using selective or elective growth medium.

2c. Whole soil as a complex test-organism can also be useful, similarly to single organisms in bioassays for ecotoxicity testing. In our laboratory we used nitrification and respiration of good quality healthy soil as endpoint for ecotoxicity testing of contaminated soil and wastes. The sterilised contaminated sample is mixed into the clean soil in growing concentration. The two ends of the series are contaminated soil and clean soil, in between the dilutions in form of soil mixture. The activity of the clean soil will be inhibited by the tested soil. ED20, ED50 or NOEL can be determined, which means

the amount of the contaminated soil, which causes 20 or 50 % decrease or *no observable effect* in the measured endpoint (nitrification, respiration, etc.). The same test may give information on the adaptive potential of the clean soil: which is proportional with the long term decrease of the inhibitory effect of the contaminant.

2d. A dynamic testing is also possible in the soil, when the steady state soil is loaded with the contaminant or any other harmful effects. The rate and type of the response of the soil and the process until the new steady state gives information about the biological state, adaptive potential, stability, biological and genetic flexibility of the soil microflora. The measured endpoints are characteristics of the whole soil, like in the previous subchapters: total or special cell concentrations, any kind of activities or special characteristics, like resistance, respiration rate, hydrocarbon degrading activity, special genes or gene-products. This kind of testing strategy overlaps in a large scale with microcosms.

3. Soil microcosms

Microcosms are multispecies systems and the tests conducted in them are generally multiparameter measurements. Total activities or any details can be measured, the soil can be sampled and the history of the microcosm can be followed. Complex procedures and interactions can be investigated in these tests. Some of them are completely artificial and controlled, but they can be a small part of the real environment. Every microcosm differs from the other, they have their own history, their ecosystem has its own evolution. This advantage is the disadvantage at the same time, because the statistics, the reproducibility is generally poor, but the response is much closer to the realty, than the simple single species bioassays.
We developed tests which measure the endpoint of respiration and/or nitrification of the soil. From samples taken from the microcosm, measuring the cell concentrations, the enzyme activities and the chemical analysis of the soil and the contaminant is possible. Microcosm tests overlap with laboratory technology experiments. Depending of the aim and strategy of the testing we can measure:

- the absolute values of soil respiration or enzyme activities,
- the changes of the activities during natural or experimental processes,
- the response on the effect of added contaminants or contaminated materials
- the behaviour of the soil during natural attenuation and enhanced attenuation
- the effect of technological parameters on bioremediation

A monitored microcosm was developed in our laboratory for modelling the pollution of soil with toxic metal containing mining waste. To follow the procedure after mixing mine waste into the soil, the following parameters were regularly measured and tested: soil physical parameters and chemical composition, like nutrient supply, humus content, Ca-content, pH, etc., contaminant content and their mobility by chemical speciation; cell concentration and respiration of the soil, toxicity of the contaminated soil on test-organisms of three trophic levels.
A small size (500–1000 g soil) microcosm for the testing of natural attenuation and enhanced bioremediation has been developed and used in our laboratory for testing biodegradability of organic contaminants, rate of biodegradation and respiration, cell concentrations and activities during the process. Also the effect of technological parameters and additives can be investigated.

The use and importance of soil ecotoxicity testing are shown by some examples:

1. Mapping of contaminated sites: the Toka-valley, the scene of a former zinc-lead mine is today a well-explored demonstration site of our environmental research (Gruiz, 2000), but in 1989, when we started with the research there, no historical documents, no information on contaminant sources, quality and quantity of the contaminants, extent of the contamination, transport routes, etc. Was available. We used ecotoxicity screening to map the toxicity of the 15 x 1 km site. We collected typical mine wastes from the site and selected a *Bacillus subtilis* bacterial strain sensitive to the contaminant mixture of the Toka-valley. The sensitivity of the test organism proved to be optimal for identifying and differentiating the risky samples of soils and sediments. Growth inhibition was measured by the fast and effective "soil diskette" method. Later on other tests and test organisms were also applied for mapping toxicity, like bioluminescence inhibition and dehydrogenase-inhibition. Toxicity mapping helped us to identify primary sources, transport routes, secondary sources and contaminated land along the Toka-valley (Gruiz and Vodicska, 1992) Chemical analysis fully proved the high level of contamination in the toxic soil and sediment samples: from 69 toxic samples all had a heavy metal contamination of 2–3 x of the limit value (Gruiz, 1994).The opposite was not true: some waste and sediment samples have not shown toxicity, but their heavy metal content by chemical analysis was much higher, that the limit value. These samples contain heavy metals in a biologically unavailable form.

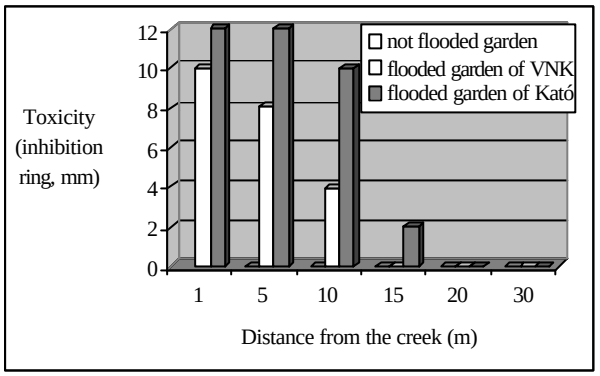

Figure 3. Toxicity of garden soils measured by the *Bacillus subtilis* "soil diskette" method

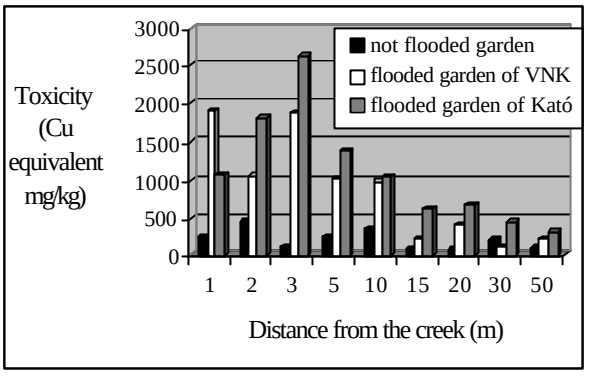

Figure 4. Toxicity of garden soils measured by *Vibrio fisheri* luminescence inhibition

On the basis of these toxicity results one of the most risky transport route was identified: transport of contaminated sediment by floods and its disposal on the soil of the hobby gardens, as it is shown on Figure 3. and 4., where the high toxicity of samples close the creek can be seen.

2. The biological availability of mine-waste pollutants is of crucial interest. Sediments, which caused the high toxicity of the hobby gardens often did not show any toxicity in spite of high metal contents measured by chemical analyses. We tried to find correlation between toxicity and total metal content (from the king's water extract) toxicity and mobile metal content (from an acetate-buffer extract) but the effects cannot be estimated on this bases, if the soils are contaminated with sediment of different origin and type. Also the time of pollution and the interactions influence toxicity.

The next two figures demonstrate two different cases: On Figure 5. plant toxicity and the amount of the chemically measured mobile fraction correlates well on soil samples from a homogeneously polluted site, but on Figure 6. the lack of association between plant toxicity and the chemical analytical results of morphologically different pollution can be seen.

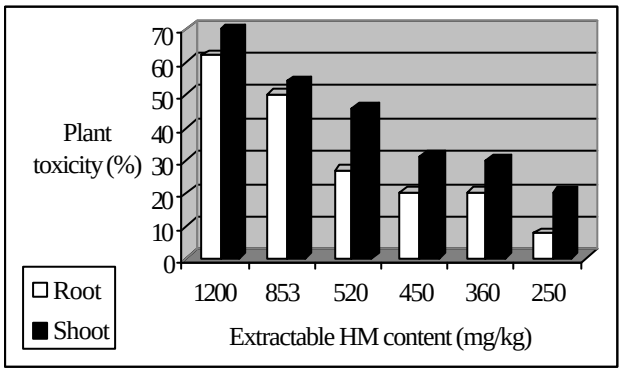

Figure 5. Plant toxicity is proportional with the amount of the mobile heavy metal fraction

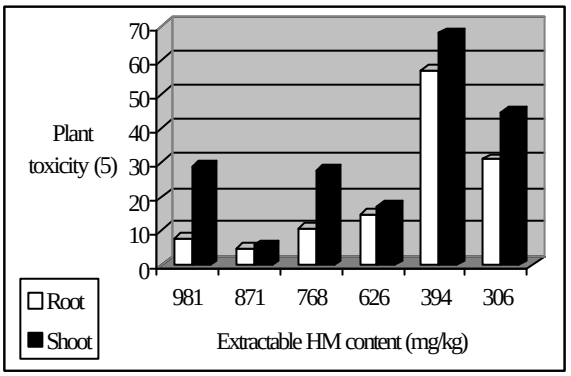

Figure 6. Plant toxicity is not proportional with the chemically measured heavy metal content

In the garden of Figure 5. yearly mild flood is typical. In the garden of Figure 6. sediments different by morphology and age (yellowish, greyish, stone like, flocculent-like, etc.) were collected and tested. Extractable heavy metal content is the sum of As, Cu, Cd, Hg, Pb and Zn. Toxicity related to single metals show the same picture.

Growing mobility is typical after the artificial pollution-incident. First the soil and the pollutant (mine waste or toxic metal containing rock and ore) were mixed with each other, when a weathering process is started. During this complicated procedure the former equilibrium of the soil is disturbed, the mobility and bioavailability of the metals increases temporary or for ever. The new equilibrium depends both on the type and characteristics of the soil and the pollutant.

Weathering and mobilisation of metals of polluted creek-sediment was tested in microcosm, modelling flood-incident. An integrated methodology was used to follow and understand the process. Chemical analyses and toxicity testing proved the mobilisation of the toxic metals after pollution. Interestingly the

weathering and mobilisation was much faster if the ratio of sediment (compared to soil) was low. It shows again, how risky the dilution can be!

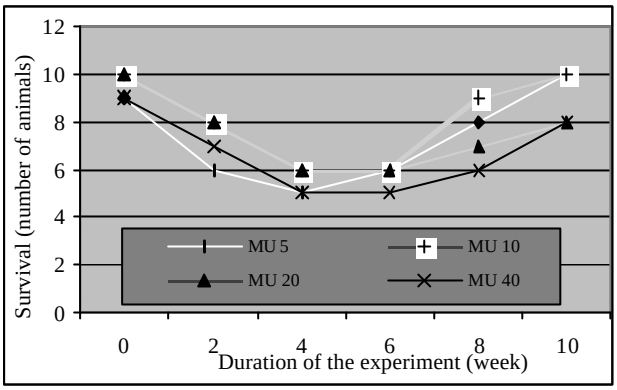

Figure 7. Changes in the survival of *Eisenia foetida* in the microcosm test modelling soil pollution with a sediment of mine waste

Figure 7. shows the changes of toxicity in the microcosm after polluting the soil with the mine-waste containing creek sediment in 5, 10, 20 and 40 %. Toxicity was measured by the earthworm (*Eisenia foetida*) acute toxicity test on samples taken from the microcosm every two weeks. Other ecotoxicity tests gave similar results and the chemical analyses proved the same: mobilisation is faster in case of the smaller contaminant concentration (MU 5%), du to faster weathering and lower pH. After a while a toxicity buffering effect is arising, the mobility and the bioavailability of the toxic metals decreases, in some cases back to the original, sometimes to a higher level, depending on the characteristics and pH of the pollutant and the soil.

In spite of the toxic-effect buffering capacity of the soil a slowly increasing toxic metal content and toxicity was measured during the long-term microcosm experiment.

The toxicity buffering character of the soil depends on the soil type and its physico-chemical characteristics. Distribution of the grain size and the mineral composition are of basic importance.

Figure 8. and 9. show the result of a bacterial bioassay measured by the same concentration of artificial metal contamination (ionic salts) in water solution, in sandy and in a loamy soil. The minimum effective level is two times more in the sandy, 4–5 times more in the loamy soil compared to the water solution. In case of non-ionic pollutants the effective toxicity in soil may be even lower compared to the solution.

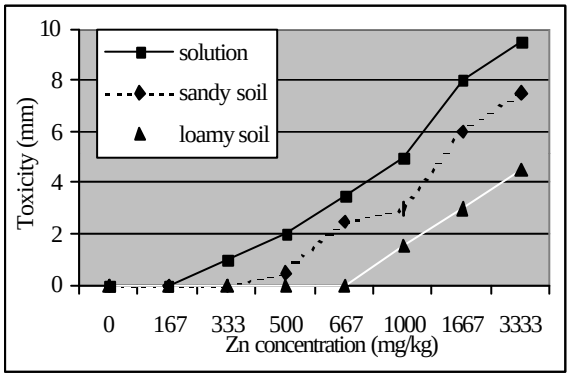

Figure 8. *Pseudomonas fluorescence* growth inhibition by the same concentration of zinc in water, in sandy and in loamy soil

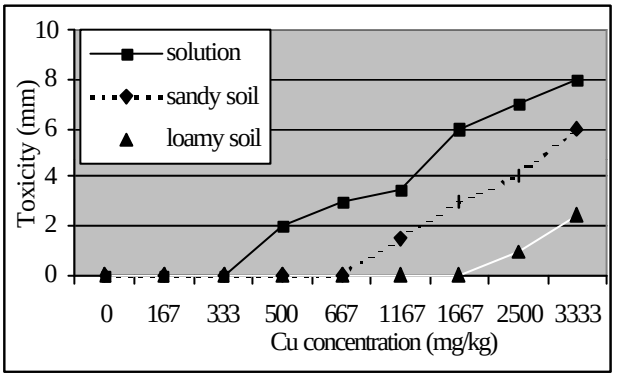

Figure 9. *Pseudomonas fluorescence* growth inhibition by the same concentration of copper in water, in sandy and in loamy soil

Monitoring of of soil bioremediation polluted with biodegradable organic contaminants needs also an integrated approach. On Figure 10. typical change of toxicity is shown in case of readily biodegradable diesel oil and moderately biodegradable (non-PCB) transformer oil. Toxicity is measured by bioluminescence-inhibition of *Vibrio fisheri*. At the beginning of the remediation the bioavailability of the contaminant increases as requirement of biodegradation. It is indicated by growing toxicity. In the course of biodegradation the concentration and in parallel the toxicity are reduced proportionally. In case of mixtures this increase followed by decrease of toxicity (and of the mobilised amount) may occur in more steps.

Figure 11. shows the changes in toxicity during the bioremediation of a coal-tar polluted soil. Bioavailability and biodegradability of coal-tar is known as being poor. In our two experiments half of the initial 20 000 and 100 000 mg/kg was eliminated in 10–15 weeks. Toxicity (which is associated with the bio-

availability) finally decreased drastically, mainly in the experiments which used an availability enhancing amendment, the cyclodextrin (Molnár, et al., 2000), but in between it was 2-3 times higher, than in the beginning. The residue, in spite of its low toxicity had a high concentration, measured after hexane–acetone extraction. It means that the residue was biologically not available.

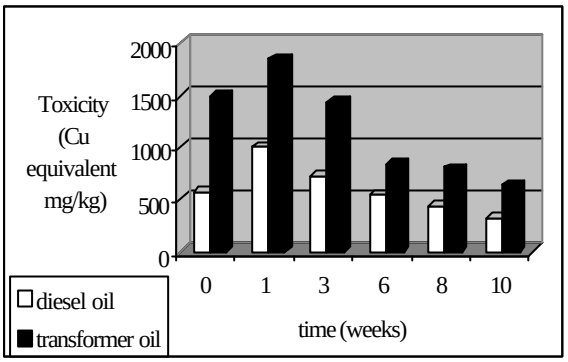

Figure 10. The change of the toxicity during bioremediation of diesel-oil and transformer-oil contaminated soil

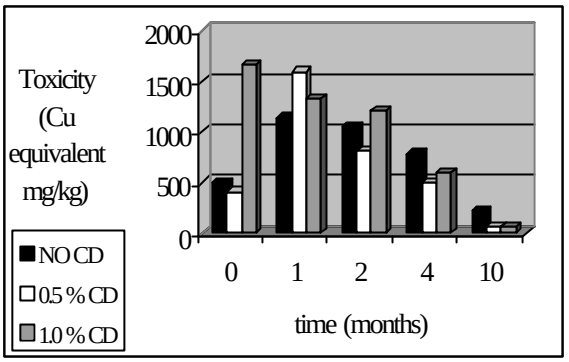

Figure 11. The change in the toxicity during bioremediation of coal-tar contaminated soil

Respiration of the soil has the widest application. It can be measured in whole soil in complete and selective nutrient media. Both the increase/stimulation and the decrease/inhibition are informative. We can indicate and quantify the existing hydrocarbon degrading activity in the soil, its changes and enhancement during the remediation, flexibility and adaptive behaviour of the soil, toxicity of the contaminants, etc.

Adaptation of the soil and biodegradation of the contaminant is shown by testing it in a soil-respirometer. The Sensomat equipment applies a closed bottle half volume filled with the tested soil, half volume air. The decrease of the pressure as a result of the consumption of O_2 by soil microflora is measured by a pressure sensing detector. It is connected to the computer software through infra-ports.

On Figure 12. typical respiration curve of a good quality soil is to be seen. The saturation curve reflects intensive respiration in the closed system. Adding toxic waste to the soil in 4:1 and 3:2 ratio, the respiration is inhibited temporarily, but thanks to the flexibility of the soil microflora after a while it recovers and makes up for lost time. But in case of a waste ratio of 1:4 not only the delay is considerable (very little respiration in the first 10 hours), but the consumed O_2 is also less, compared to the untreated.

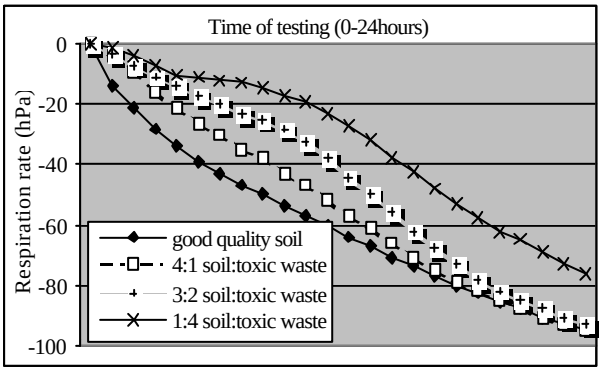

Figure 12. Respiration curves of soils without and with toxic additives

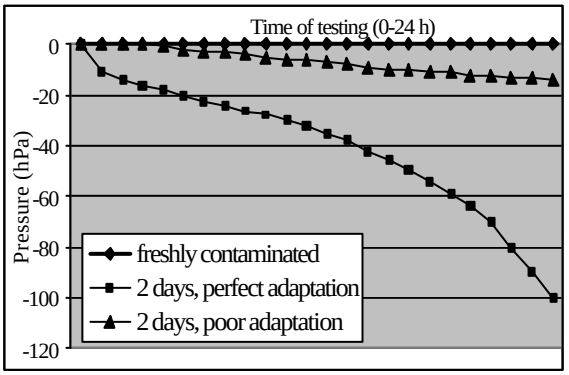

Figure 13. Inhibition of soil respiration by adding biodegradable hydrocarbons to the soil

In Figure 13. a mixture of diesel and engine oil is added to the soil. Immediately after the artificial contamination the soil respiration is completely inhibited. After 2 days adaptation period the good quality soil with a flexible microflora and with a good adaptive behaviour results in a curve, which shows the start of the normal respiration (which would be a saturation curve) plus an increase from the

20^{th} hour due to the biodegradation of the hydrocarbon. For the bad quality soil 2 days adaptation period was not enough to recover and become able to biodegrade the contaminant.

Conclusion

An integrated approach is necessary in environmental assessment and monitoring of contaminated sites, natural attenuation and remediation technologies. The integrated approach means that physico-chemical analyses should be complemented with biological measurements and ecotoxicity testing to get additional information about bioavailability, about not measured or not measurable but dangerous components, about the background history of the soil biota.

Toxicity testing of soil is relatively new, special test methods are required because of the specificity of the soil: the three phases, the non-equilibrium state, the flexible and continuously changing, adapting microflora, etc. Test methods and test batteries are needed to characterise contaminated soil as a dynamic system, which is able to measure responses and interactions.

In the first part of the paper an overview is given on those tests, which are suitable for testing whole soil, the effects of the contaminant on the soil itself and its ecosystem. Three type of tests were developed and used in our environmental research: 1. direct contact single cell laboratory bioassays with microbes, plants and animals, 2. whole soil tests measuring cell concentrations, diversities and activities of the soil, like respiration, enzyme activities, adaptation, biodegradation, resistance, etc. and 3. soil microcosm modelling the real soil system according to the aim of testing, from multispecies ecotoxicity assessment to the technological experiments.

Application and getting amended information by the use of ecotoxicity testing is demonstrated by a number of examples from the practice of the environmental microbiology research of BUTE, both from the area of inorganic and organic contaminants.

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