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Comparison of bioassays by testing whole soil and their water extract from contaminated sites

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Abstract

The harmful effects of contaminants on the ecosystems and humans are characterised by their environmental toxicity. The aim of this study was to assess applicability and reliability of several environmental toxicity tests, comparing the result of the whole soils and their water extracts. In the study real contaminated soils were applied from three different inherited contaminated sites of organic and inorganic pollutants. The measured endpoints were the bioluminescence inhibition of *Vibrio fischeri* (bacterium), the dehydrogenase activity inhibition of *Azomonas agilis* (bacterium), the reproduction inhibition of *Tetrahymena pyriformis* (protozoon), and *Panagrellus redivivus* (nematode), the mortality of *Folsomia candida* (springtail), the root and shoot elongation inhibition of *Sinapis alba* (plant: white mustard) and the nitrification activity inhibition of an uncontaminated garden soil used as ''test organism''. Besides the standardised or widely used methods some new, direct contact ecotoxicity tests have been developed and introduced, which are useful for characterisation of the risk of contaminated soils due to their interactive nature.

Soil no. 1 derived from a site polluted with transformer oil (PCB-free); Soil no. 2 originated from a site contaminated with mazout; Soil no. 3 was contaminated with toxic metals (Zn, Cd, Cu, Pb, As).

In most cases, the interactive ecotoxicity tests indicated more harmful effect of the contaminated soil than the tests using soil extracts. The direct contact environmental toxicity tests are able to meet the requirements of environmental toxicology: reliability, sensibility, reproducibility, rapidity and low cost.

Keywords: Environmental toxicity; Interactive test methods; Risk assessment; Transformer oil; Mazout; Heavy metals

1. Introduction

Chemical data alone are not sufficient to evaluate the toxic effects of the contaminants and characterise contaminated environment, because they are not able to provide information on the effects of the chemical compounds and do not take into consideration the interactions between contaminants, matrix and biota. To estimate the risk of contaminants and contaminated environmental elements, chemical methods have to be complemented with biological and toxicological methods (Joergensen et al., 1995; Margesin et al., 2000; Gruiz et al., 2001; Monkiedje et al., 2002; Vaajasaari et al., 2002; Fent, 2003; Tsui and Chu, 2003; Robidoux et al., 2004; Gruiz, 2005; Molnár et al., 2005). The integrated methodology gives information not only about the quality and quantity of the contaminants, but also about the effects of the soil, the biological status, the degradative activity of the soil microflora as well as about the interactions between all of the soil components: the contaminants, the three soil phases, and the different members of the soil biota. Ecotoxicity tests measure the bioavailability of the contaminants and the effects of the chemically not measured toxic compounds on the members of the soil community (Gruiz, 2005). The integrated

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approach is used for site assessment (Gruiz et al., 1998a), for the risk-based management of contaminated sites, including the establishment of target quality criteria, for selection of the best possible technology and for monitoring (Dorn and Salanitro, 2000; Juvonen et al., 2000; Leitgib et al., 2003; Molnár et al., 2005).

The risk of the contaminants on soil and ground water is estimated from the test-results of soil extracts (Hammel et al., 1998; Bispo et al., 1999; van Gestel et al., 2001; Robidoux et al., 2004). Some standards recommend aquatic organisms for testing of ground water or soil leacheates: these results are not relevant for them out and away for the surface waters polluted by transport of the contaminant from the soil. To characterise the soil as habitat or as element used by humans, the so called direct contact environmental toxicity tests have been recently developed and applied (Gruiz et al., 1998b; Hammel et al., 1998; Juvonen et al., 2000; Abbondanzi et al., 2003), which allow to manifest all kind of interactions between the contaminants, soil matrix and the test organism and give more realistic results integrating all site specific effects (Gruiz, 2005). The effect of pollution on the soil ecosystem should be characterised by the use of selection of species representing the whole ecosystem. To find the relevant species a good scientific background is necessary, because difference in sensitivity within the same taxa is also usual (Heupel, 2002; Sverdrup et al., 2003).

The aim of our present study was to measure the sensitivity of several standardised and self developed environmental toxicity tests to three soils originated from contaminated sites and to prove the application of the direct contact between the soil and the test organism to simulate real situations and actual risk of the soil as habitat. Enzyme activities of microbial cells, protozoon, animals and plant organisms were used to measure and compare the effects of selected contaminated soils.

2. Materials and methods

2.1. Soil samples: Origin and physico-chemical properties

Soils originated from actual sites polluted with various contaminants.

Soil no. 1 is a transformer oil-contaminated soil. The source of the contamination is a leaking spare transformer. The pollution is about 10 years old; the oil is PCB-free. During site assessment of the transformer station an integrated methodology was applied: hydrogeological assessment of the site, characterisation of the soil by the use of chemical analyses of the contaminants and environmental toxicity testing of the soil samples.

Soil no. 2 is a soil contaminated with mazout. Origin of the pollution is mazout storage in non-sealed basins. Mazout is the residual product of petroleum refining. It contains heavy, high molecular weight hydrocarbons, polycyclic aromatic hydrocarbons and phenolic compounds. In the sixties and seventies it was used as heating oil that is why it was stored and commercially applied in large quantities. The mazout-contaminated soil was sampled and analysed after excavation and dumping.

Soil no. 3 was taken from a former Zn and Pb mining site, contaminated with acid mine drainage and mine wastes, which contained several heavy metals, mainly Zn, Cd, Cu, Pb and As. As part of the integrated methodology, environmental toxicity of the contaminated soil was tested.

The physico-chemical properties of the tested soils are given in Table 1.

The water-soluble salts and the $CaCO_3$ content were determined according to the Hungarian standard (HS 08-0206-2). Organic carbon content was determined by oxidation with potassium dichromate in an acid medium and measurement using a spectrophotometer. For determination of the pH 1 g air-dried soil was suspended with distilled

Table 1
Physico-chemical characteristics of the uncontaminated garden and the contaminated soils and concentrations of the contaminants

Parameter	Soil no. 1	Soil no. 2	Soil no. 3	Uncontaminated garden soil
pH	8.0	7.5	3.7	6.8
All water soluble salts content (%)	<0.02	0.16	<0.02	<0.01
$CaCO_3$ content (%)	6.0	5.0	0.0	7.0
Humus content (%)	1.8	4.1	1.6	4.7
EOM (mg kg^{-1})	49400	6100	n.m.	n.m.
EPH (mg kg^{-1})	30700	3900	n.m.	n.m.
Total Zn content (mg kg^{-1})	n.m.	n.m.	1034.8	n.m.
Soluble Zn content (mg kg^{-1})	n.m.	n.m.	267.6	21.3
Total Cd content (mg kg^{-1})	n.m.	n.m.	8.4	n.m.
Soluble Cd content (mg kg^{-1})	n.m.	n.m.	1.7	0.1
Total Cu content (mg kg^{-1})	n.m.	n.m.	336.6	n.m.
Soluble Cu content (mg kg^{-1})	n.m.	n.m.	17.1	9.2
Total Pb content (mg kg^{-1})	n.m.	n.m.	1497.4	n.m.
Soluble Pb content (mg kg^{-1})	n.m.	n.m.	35.4	6.9
Total As content (mg kg^{-1})	n.m.	n.m.	357.9	n.m.
Soluble As content (mg kg^{-1})	n.m.	n.m.	<0.5	<0.5

n.m.: not measured. EOM: extractable organic material content. EPH: extractable petroleum hydrocarbon content.

water at a ratio of 1:2.5 (w/v) and was measured with a pH-electrode after shaking at 220 rpm for 30 min and filtration. Extractable organic material (EOM) content was measured by gravimetry after extraction of 5 g of soil with hexane–acetone (2:1). The extractable petroleum hydrocarbon content (EPH) was quantified by GC-FID according to the EPA 8270 and HS 21470-94. Heavy metal contents of the Soil no. 3 and the uncontaminated garden soil were analysed by inductively coupled plasma after acid digestion with HNO_3:H_2O_2 in a ratio of 5:2 (v/v) at 105 °C for 3 h.

2.2. Preparation for testing

For environmental toxicity testing soils were homogenised, air-dried and sieved (<2 mm). For soil extraction 40 g of the soil sample was suspended in 80 ml deionised water. The mixture was shaken at 220 rpm for 4 h and filtered on 0.45 μm filter. The eluates were stored at 4 °C until testing.

2.3. Environmental toxicity testing from whole soil samples

Most of the test procedures were developed by our laboratory or modified from one of the standard proposals (ISO/DIN/HS). The artificial OECD soil was used as uncontaminated control soil for the *Vibrio fischeri* bioluminescence inhibition test, the *Folsomia candida* mortality test, the *Sinapis alba* root and shoot elongation test and was prepared according to OECD Guideline 207 (1984). Because OECD soil had adverse effect on the test organism, an uncontaminated garden soil was used as control soil for the *T. pyriformis* reproduction inhibition test. It was also used as "test organism" for the nitrification activity inhibition test due to its high nitrification activity.

Environmental effect of soils on the *V. fischeri* (NRRL B-111 77) was determined by the bioluminescence inhibition test. The light production of the test bacterium was measured by a luminometer (Lumac Biocounter M 1500 l). 2 g of soil samples was suspended in 2 ml 2% NaCl solution. A five-step dilution series was prepared from the contaminated soils. After measurement of the reference luminescence intensity, 50 μl of the dilution series was added to the test medium. The luminescence intensity was repeatedly measured after 30 min exposure time.

Azomonas agilis bioassay is based on the dehydrogenase activity inhibition caused by toxic effect of the soil. 100 ml sterile medium was supplemented with 1 ml 1% 2,3,5-triphenyl tetrazolium chloride (TTC) as an artificial electron acceptor and with the test bacteria previously incubated on a rotary shaker at 28 ± 2 °C for 72 h. The stock solution was injected into the tubes that contained the dilution series of the contaminated soils (dilution factor 2). The serial dilutions were incubated at 28 ± 2 °C for 72 h in the dark. TTC is reduced by microbial activity to red-coloured formasan, which was determined visually.

T. pyriformis reproduction inhibition test characterises the toxic effect of the contaminated soils on a protozoon, a primary consumer in the food chain. *T. pyriformis* A-759-b was grown in proteose peptone yeast extract medium (PPY), containing 1% proteose peptone and 0.1% yeast extract. The test tubes containing 0.25 g of sterile soil were supplemented with 5 ml of PPY medium and with Penicillin-, Streptomycin- and Nystatin solutions at final concentration 0.01 mg ml^{-1}, 0.1 mg ml^{-1}, 0.005%, respectively. After vigorous mixing 100 μl of six-day-old test organisms (about 1000 cells/ml) were added to the tubes. During incubation period (20 ± 2 °C, 250 rpm) sampling was carried out six times (0, 22, 46, 65, 73, and 88 h). The actual cell concentration was determined by direct counting in Bürker counting chamber.

F. candida mortality test measures the toxic effects of the contaminated soils on a soil animal. A two-fold dilution series was prepared from the contaminated soil samples with OECD soil at final concentrations from 100% to 6.25%. Ten pieces of twenty-day-old springtails from a synchronized culture were transferred into the test flasks (250 ml) containing 20 g wet mass of the soil mixtures. The soil mixtures were moistened with 9 ml of water. The springtails were fed with commercial lyophilised bakers'yeast. Test flasks were incubated 20 ± 2 °C in the dark for 14 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.

S. alba as representative of plants was used for the plant assay. A five-step two-fold dilution series was prepared by mixing the contaminated and the OECD soil. 5 g wet mass of the soil mixtures was taken into 10 cm diameter Petri dishes and brought to similar moisture content with water. 20 seeds with >90% germination ability were arranged on the soil surface. The test dishes were kept in the dark at 20 ± 2 °C for 72 h. The length of the grown root and shoot was measured with ruler.

Nitrification activity inhibition test measures the potential ammonium oxidation activity of the soil. It is a further development of the method worked out by Berg and Rosswall (1985). The 100% nitrification activity was ensured by an uncontaminated garden soil and the decrease in this high activity is caused by the contaminated soils. A five-step dilution series was prepared from the mixture of the contaminated and the garden soils. 5 g of the soil mixture was suspended in 0.1 ml $NaClO_3$ (1.5 M) and 20 ml $(NH_4)_2SO_4$ (1 mM) solutions. Test flasks were incubated on a rotary shaker (220 rpm) at 28 ± 2 °C for 5 h. To determine the initial concentration of nitrite, the soil mixtures were suspended in distilled water instead of $(NH_4)_2SO_4$ at −20 °C for 5 h. At the end of the incubation period, the nitrification process was terminated by addition of 5 ml 2 M KCl. After centrifuging (4500 rpm, 2 s) samples were sieved on N-free filter. The nitrite content was measured by photometry at 538 nm.

2.4. Environmental toxicity testing from soil extracts

V. fischeri bioluminescence inhibition test was used to evaluate the toxicity of the soil eluates. The parameters

of the test were similar to the above described bioluminescence test but the measurement was going on in water solution with soil eluates instead of soil suspension.

The *A. agilis* dehydrogenase activity test is often used for toxicity testing of extracts of wastes and soils to model the risk of contaminated ground water. The bioassay was conducted in microtiter plate according to the Hungarian standard (HS 21978-30). 0.05 ml of the dilutions of the soil eluates were mixed into 0.2 ml of the test medium. It consists of 100 ml nutrient medium, 1 ml 1% 2,3,5-triphenyl tetrazolium chloride (TTC) as an artificial electron acceptor and the test-bacteria. The microtiter plate was covered and incubated at 28 ± 2 °C for 48 h in the dark. The appearance of the red colour was indicated the microbial activity and evaluated visually.

The protozoon bioassay applied 72 h cultures of *T. pyriformis* A-759-b grown in Proteose Peptone Yeast Extract Medium (PPY). The initial cell density of the test suspension was 60 cells ml^{-1} in the medium. Conventional 96-well microtiter plate was used for the bioassay. Test medium (0.18 ml) was added into each well and amended with 0.02 ml of the contaminated soil extracts. Sterile distilled water was used as a control. The outer wells were filled with sterile distilled water to prevent evaporation. The plate was covered and incubated in the dark at 20 ± 2 °C for four days without shaking. The cell density in wells was evaluated under a microscope with 4-fold magnification.

Panagrellus redivivus nematode reproduction inhibition test investigated eluates of the contaminated soils in conventional 96-well microtiter plate. Eight parallel wells were filled with 0.8 ml of liquid nutrient medium and two 12-day-old nematodes were added. The test container was covered and incubated in the dark at 20 ± 2 °C for one week. 0.2 ml of the soil eluate and nutrient medium was added into the wells, when the first generation appeared in the most of the test wells. After further one-week incubation, the second generation could be investigated in the wells including the soil extracts. The cell density could be observed with microscope (4-fold magnification).

S. alba (mustard-seed) assay was modified from the Hungarian standard (HS 21978-8). Ten centimeter diameter Petri dishes were used for the testing. After placing <5 mm pore filters into the dishes, 3.5 ml of the contaminated soil extract was injected in increasing concentrations (dilution factor 2). Twenty seeds of similar size and colour were arranged at the surface of the filter papers. The test dishes were placed in the dark at 20 ± 2 °C for 72 h. The length of the germinated root and shoot was measured with ruler.

2.5. Evaluation and interpretation of the results

Evaluation and interpretation of the ecotoxicity tests are summarised in Table 2.

ED_{50} (LD_{50}) means contaminated soil dose that caused 50% inhibition (lethality) in the endpoint of the tests. For better interpretation of the results we worked out the "Copper Equivalent Method" for the soil sample containing toxic metals. The 50% inhibition of Soil no. 3 is given in Cu equivalent ($\sum Cu_{50}$ (mg Cu kg^{-1} soil)), which means the Cu concentration that would cause the same toxicity as the actual pollution in the soil. The concentration of Cu equivalent is determined from the Cu-calibration curve considering the same % of inhibition or other effect of the soil. To help understanding of the results of the tests with different sensitivity we characterised the samples as: "non-toxic", "slightly toxic", "toxic" and "very toxic".

T-test was used for independent samples to evaluate the differences between the response of the test organisms in the control and the contaminated soils. Significant differences were accepted at $p < 0.05$. Statistical analyses were performed by the use of StatSoft® Satistica 6.0 program.

Table 2
Evaluation and interpretation of the bioassays

Endpoint of the measurement – inhibition	Contact	Evaluation of the tests	Interpretation of the results	Characterisation
Vibrio fischeri bioluminescence	Direct contact	Origin 6.0	ED_{50} (g soil)[a]	Scale of toxicity
	Water extract	Origin 6.0	ED_{50} (g soil)[a]	Scale of toxicity
Azomonas agilis dehydrogenase activity	Direct contact	Semi-quantitative (colour changing)	Inhibition (%)	Scale of toxicity
	Water extract	Semi-quantitative (colour changing)	Inhibition (%)	Scale of toxicity
Tetrahymena pyriformis reproduction	Direct contact	Origin 6.0	Inhibition (%)	Scale of toxicity
	Water extract	Semi-quantitative (density)	Inhibition (%)	Scale of toxicity
Folsomia candida mortality	Direct contact	Origin 6.0	LD_{50} (g soil)[a]	Scale of toxicity
Panagrellus redivivus reproduction	Water extract	Semi-quantitative (density)	Inhibition (%)	Scale of toxicity
Sinapis alba root and shoot elongation	Direct contact	Origin 6.0	ED_{50} (g soil)[a]	Scale of toxicity
	Water extract	Origin 6.0	ED_{50} (g soil)[a]	Scale of toxicity
Nitrification activity	Direct contact	Origin 6.0	ED_{50} (g soil)[a]	Scale of toxicity

ED_{50}: soil doses that cause 50% inhibition.
[a] Effect of soil no. 3 is also given in Cu equivalent, $\sum Cu_{50}$ (mg Cu kg^{-1} soil).

3. Results and discussion

Effects of the contaminated soils on the test organisms were measured and compared to the OECD soil or the uncontaminated garden soil used as control, respectively. Table 3 summarises the toxicity results of the bioassays.

Direct contact tests using microbial test organisms (*V. fischeri* and *A. agilis*) were very sensitive to all of the contaminated soils (Table 3). Transformer oil-, mazout- and heavy metal-contaminated soils inhibited especially the bioluminescence production of *V. fischeri* in soil suspension. In case of transformer oil the luminescent bacterium was inhibited by soil eluates as well. In the other two cases toxic effect was not measured. Moreover, none of the soil extracts showed toxicity on the dehydrogenase activity of *A. agilis*.

In the *T. pyriformis* direct contact test the inhibition was calculated from the specific reproduction rate, which is constant in the exponential growth phase. Cell concentration of the protozoon in the uncontaminated garden and the contaminated soils during the exposure time in the contact test are summarised in Table 4.

As a result of the direct contact all soil suspensions inhibited the reproduction of the *T. pyriformis*. However, only the mazout-contaminated soil extract was toxic to the protozoon.

According to the *F. candida* mortality test all contaminated soils had a toxic effect on soil animals. Neither in oil-contaminated nor in heavy metal contaminated soils any adults survive.

Both eluates of the transformer oil- and the mazout-contaminated soil inhibited the reproduction of *P. redivivus*.

Table 3
Toxic effects of the contaminated soils on the test organisms in the ecotoxicity tests

Endpoint of the measurement – inhibition	Contact	Interpretation of the results	Results and characterisation of the soil no. 1		Results and characterisation of the soil no. 2	
Vibrio fischeri bioluminescence	Direct contact	ED_{50} (g soil)	0.02	Very toxic	0.03	Very toxic
	Water extract	ED_{50} (g soil)	0.01	Very toxic	$\gg 0.02$	Non-toxic
Azomonas agilis dehydrogenase activity	Direct contact	Inhibition (%)	74	Toxic	94	Very toxic
	Water Extract	Inhibition (%)	0	Non-toxic	0	Non-toxic
Tetrahymena pyriformis reproduction	Direct contact	Inhibition (%)	100	Very toxic	34	Toxic
	Water extract	Inhibition (%)	0	Non-toxic	70	Very toxic
Folsomia candida mortality	Direct contact	LD_{50} (g soil)	13.1	Toxic	12.5	Toxic
Panagrellus redivivus reproduction	Water extract	Inhibition (%)	93	Very toxic	83	Very toxic
Sinapis alba						
Root elongation –shoot elongation	Direct contact	ED_{50} (g soil)	2.4	Toxic	>5	Non-toxic
			1.2	Toxic	>5	Non-toxic
	Water extract	ED_{50} (g soil)	0.4	Very toxic	>5	Non-toxic
			0.5	Very toxic	>5	Non-toxic
Nitrification activity	Direct contact	ED_{50} (g soil)	1.6	Toxic	2.5	Toxic

Endpoint of the measurement – inhibition	Contact	Interpretation of the results	Results of the soil no. 3	Interpretation of the results	Results and characterisation of the soil no. 3	
Vibrio fischeri bioluminescence	Direct contact	ED_{50} (g soil)	0.03	$\sum Cu_{50}$ (mg Cu* kg^{-1} soil)	786	Very toxic
	Water extract	ED_{50} (g soil)	$\gg 0.02$	$\sum Cu_{50}$ (mg Cu* kg^{-1} soil)	$\ll 120$	Non-toxic
Azomonas agilis dehydrogenase activity	Direct contact	Inhibition (%)	92	n.m.	n.m.	Very toxic
	Water extract	Inhibition (%)	0	n.m.	n.m.	Non-toxic
Tetrahymena pyriformis reproduction	Direct contact	Inhibition (%)	39	n.m.	n.m.	Toxic
	Water extract	Inhibition (%)	20	n.m.	n.m.	Slightly toxic
Folsomia candida mortality	Direct contact	LD_{50} (g soil)	13.8	$\sum Cu_{50}$ (mg Cu* kg^{-1} soil)	n.m.	Toxic
Panagrellus redivivus reproduction	Water extract	Inhibition (%)	6	n.m.	n.m.	Non-toxic
Sinapis alba root elongation –shoot elongation	Direct contact	ED_{50} (g soil)	4.3	$\sum Cu_{50}$ (mg Cu* kg^{-1} soil)	29	Slightly toxic
			4.6		30	Slightly toxic
Nitrification activity	Water extract	ED_{50} (g soil)	2.1	$\sum Cu_{50}$ (mg Cu* kg^{-1} soil)	59	Toxic
			1.6		86	Toxic
	Direct contact	ED_{50} (g soil)	2.7	$\sum Cu_{50}$ (mg Cu* kg^{-1} soil)	2669	Toxic

n.m.: not measured.

Table 4
Concentration of *Tetrahymena pyriformis* (*10^4 cell g^{-1} soil) in the uncontaminated garden and the contaminated soils during the contact testing

Exposure time (h)	Uncontaminated soil	Soil no. 1	Soil no. 2	Soil no. 3
0	2.4 ± 0	2.4 ± 0	2.4 ± 0	2.4 ± 0
22	18.2 ± 2.5	2.4[a] ± 0	17.0 ± 3.2	14.6[a] ± 0.6
46	239.6 ± 81.0	2.0[a] ± 0.7	231.3 ± 45.4	212.6 ± 80.7
65	577.1 ± 35.0	1.6[a] ± 0.7	386.1[a] ± 14.0	389.7[a] ± 20.7
73	695.8 ± 38.8	1.6[a] ± 0.7	400.9[a] ± 49.5	421.2[a] ± 44.2
88	712.5 ± 186.0	1.2[a] ± 0	564.9 ± 3.4	551.7 ± 350.4

Data are expressed as mean ± standard deviation.
[a] Significant differences between groups ($p < 0.05$).

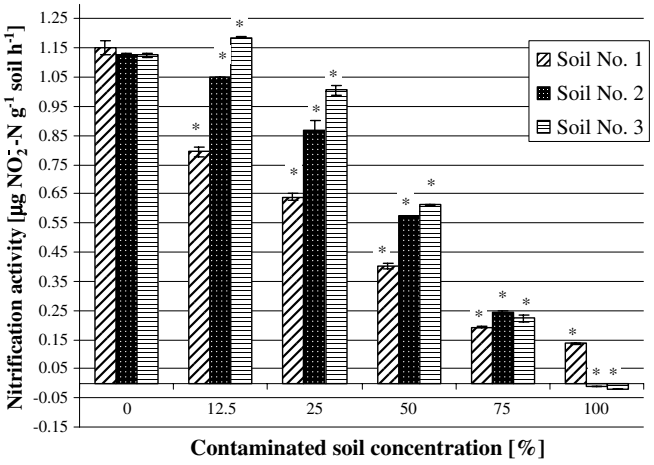

Fig. 1. Effect of contaminated soils on the nitrification activity (µg $NO_2^- - N$ g^{-1} soil h^{-1}) of the uncontaminated garden soil. Vertical bars indicate ± standard deviations. * represents significant differences between groups ($p < 0.05$).

After exposure juveniles were found in heavy metal-contaminated soil extract, indicating lower sensitivity of the test to heavy metal-contaminated soils.

The mustard-seed gave soil-dependent results according to the three different contaminants. The specific response of plant differs from other test organisms': soil extracts had slightly greater inhibitory effects than whole soils contaminated with transformer oil or heavy metals. Soil no. 2 was not toxic to the mustard seeds.

Nitrification activity of the soil microflora was also used to characterise the risk of the contaminated soils. Nitrification activity (µg $NO_2^- - N$ g^{-1} soil h^{-1}) of the mixture of the contaminated soils and the uncontaminated garden soil can be seen in Fig. 1.

All of the three soil samples inhibited the nitrification activity of the uncontaminated garden soil.

4. Conclusion

In this study environmental toxicity methods were compared for three different contaminated soils originated from polluted sites. The test organisms of the bioassays were: two bacteria, one plant, one protozoon, two animals and the nitrification activity of an uncontaminated garden soil. Both of the whole soil and the soil extract were tested. Besides the standardised or widely used methods some innovative, interactive e.g., direct contact toxicity tests have been developed and applied for the solid phase environmental samples.

The various bioassays showed different sensibility of the contaminated soils. In the direct contact tests all test organisms except *A. agilis* indicated the highest toxicity of Soil no. 1 contaminated with transformer oil. The high oil concentration (EPH 30 700 mg kg^{-1} soil) resulted in the high toxicity.

Although the mazout was not present in large quantities in Soil no. 2 (EPH 3 900 mg kg^{-1} soil), it resulted in high toxicity: the highest inhibition was measured in the direct contact tests with microbial test organisms. The extract of this soil was also very toxic to the reproduction of *T. pyriformis* and of *P. redivivus*. Medium toxicity was measured by the nitrification activity test and by the protozoon and animal test organisms. White mustards were not sensitive for mazout-contaminated soil in this range of the concentration.

Soil no. 3 contaminated with toxic metals inhibited the bioluminescence production of *V. fischeri*, the dehydrogenase activity of *A. agilis*, the reproduction of *T. pyriformis*, the surviving of the *F. candida*, the germination of the mustard and the nitrification activity. Even if the Soil no. 3 was intensively vegetated on site, the toxicity tests showed toxic effects therefore we can suppose high risk at this site.

According to our results it is highly recommended to complement the chemical analyses with environmental toxicity testing to characterise the risks of contaminated soils. In addition, the results of the bioassays show clearly that the test results of soil extracts predict the risk of the contaminants on ground water but not on whole soil as an environmental element. The main advantage of the direct contact is that interaction occurs between soil and test organisms, so that the mobility and bioavailability of the contaminant is included into the result. All new methods are able to meet the requirements of environmental toxicology: reliability, sensitivity, reproducibility, rapidity and low cost.

Our further aim is to develop a practical interpretation for organic contaminants similarly to the "Copper Equivalent Method" used for toxic metals to express toxic effects in concentration and use this result for quantification of the risk.

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