IVL-REPORT

Microtox Toxicity in Soil

Evaluation of the Direct Contact Test with Luminiscent Bacteria

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Sammanfattning/Summary

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List of Content

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Sammanfattning

Effekter av jord undersöktes i test baserat på bakteriell luminiscens med *Vibrio fischeri* med organismer i direkt kontakt med jordpartiklar. Tillämpningen på jordprov medförde problem med avskiljningen av jordpartiklar före ljusintensitetsmätningen. Närvaro av partiklar vid ljusmätningen påverkade ljusintensiteten genom såväl ljusabsorption som ljusspridning. Dessa bidrag kompenserades för dels genom ett omarbetat avskiljnings-förfarande utarbetat för test av sediment, dels genom korrektionsmätning i en tvåkam-markyvett för resterande partiklars bidrag.

Nitton prover av jord av olika ursprung, naturliga okontaminerade, förorenade, eller avsiktligt tillförda toxiska ämnen har undersökts. Effekter från <10-590 toxiska enheter (TU)/g torr jord erhölls. Effekten i jordprov från kontrollokaler varierade beroende på innehållet organiskt material och jordens struktur och övriga sammansättning. Starkt förorenade jordprover gav hög effekt, men i några fall oväntat låg effekt, vilket tolkades som resultat av varierande tillgänglighet. Fastän elutriat av jord gav genomgående lägre effekter (i vissa kontrollokaler var jorden helt utan spårbar effekt) var dessa väl korrelerade med test med direkt exponering av partiklar för organismer.

1. Introduction

Acute toxic effects of soils have earlier been tested with the bacterial luminescence of *Vibrio fischeri* as elutriates with aqueous solutions (1-3). The toxicity test utilises the inhibitory action of toxic substances on the bioluminescence, and a commercial test has been introduced under the trade name Microtox®. Light is produced in a luciferin-luciferase system linked to the cellular energy transfer (4), and inhibitory actions at different levels in this process will interfere with the production of light. Test of elutriates will examine a fraction of soluble substances. Recently, a method using the same test organism was developed to testing in direct contact with particulate material (5,6). This way of testing solid samples could also assess the effects of substances associated with particulate material. The method was applied to testing of sediments (7,8).

The objective was to adopt the sediment test procedure to soil samples and evaluate toxicity data in relation to physical, chemical, and other toxic parameters in soil.

2. Materials and Methods

2.1 Sampling sites

Reference soil from a site with no known contamination was obtained from an area in northern Finland, Martiniemi 30 km N Oulu. One garden soil and a commercial peat-based soil were also used as references. A spruce forest soil sample was obtained from a forest with no known contamination. Contaminated soils were obtained from a soil remediation project in northern Finland, Haukipudas and Oulainen, close to Oulu. These soils originated from industrial wood preservation sites and contained pentachlorophenol and other chlorophenolic substances (9-12). A third chlorophenol containing contaminated soil was obtained from Toras, a saw-mill with previous pentachlorophenol wood preservation.

2.2 Pretreatments of samples

Samples were stored in closed containers at -20°C prior to testing. Dry weights were determined by drying 18 h at 105°C. Content of organic material in the soils (LOI) was determined as the percentual loss in dryweight on ignition 2 h at 550°C.

2.3 Materials

We used plastic test tubes, volume c. 5 ml, with a filtering device especially designed for this purpose (Microbics Corp., Carlsbad, USA). The filter device was in the form of a plunger with a filter disc in its bottom. By pressing the plunger into the test tube the filtrate passed through the filter disc into the open interior of the plunger. From this inner compartment the filtrate could be transferred for luminometric measurements.

2.4 Test performance

2.4.1 Preparation of soil suspension

Soil (3.0 g) was suspended in 30 ml 2 % sodium chloride and pH was adjusted to 7.3 with NaOH or HCl.

2.4.2 Pretest for selection of soil concentrations in the main test

2.4.2.1 Preparation of a dilution series of soil suspension

Seven 5 ml plastic test tubes (Microbics) were placed in a thermostatted waterbath (Heto Birkeröd, 02 PT 623) thermostatted at 15 ± 0.1°C. To six of the tubes 1.5 ml of 2% sodium chloride was added. To the seventh tube 1.5 ml of the soil suspension (2.4.1) was added. Another 0.15 ml of the concentrated soil suspension was mixed with the 1.5 ml 2 % sodium chloride in one of the other tubes. The tube was shaken and 0.15 ml thus diluted suspension was transferred to the next tube. The procedure was repeated to the following tube and the one following that. After mixing of the latter, 0.15 ml of this suspension was discarded. Three tubes contained only 2 % sodium chloride (blank tubes). The instrumentation (Microtox M 500 Toxicity Analyzer, Microbics Corp. Carlsbad, USA) allowed three samples of soil in totally 15 test tubes to be tested in this pretest at the same time.

The samples of the dilution series were thermostatted 10 min at 15°C.

2.4.2.2 Addition of bacteria

Lyophilized bacteria were suspended according to the manufacturer's instructions (1). Equilibration and temperature adjustment was allowed for 15 min. Twenty µl suspension of bacteria was added to the soil suspensions and blanks with 15 sec intervals. The filter plungers were pressed through the tubes down to just above the surface of the suspension and the mixtures were shaken.

2.4.2.3 Incubation

The contact time between bacteria and soil particles was 30 min. Some times after the addition the mixtures were gently shaken, but during the last period (at least 5 min) the suspensions were allowed to settle to facilitate the separation of bacteria and soil particles.

2.4.2.4 Filtration of soil particles

After 30 min the filter plunger was pressed through the tubes with 15 sec interval between each tube. A little more than the top two thirds of the suspension was pressed through the filter. The sedimented part of the incubated suspension was not filtered.

2.4.2.5 Light intensity measurements

One ml of each filtrate was transferred to cylindrical glass cuvettes and were temperature adjusted in the thermostate of the instrument (Microtox M 500 Toxicity Analyzer, Microbics Corp., Carlsbad, USA). Five min after cessation of the incubation the light intensity of blanks and samples were read with 15 sec intervals. The analyser was connected to the serial interface of a PC (Copam) and the raw light intensity data printed by a program written in Microsoft Quick Basic.

2.4.2.6 Calculations and evaluation of the pretest

We used a Microsoft Excel spread sheet for calculations. A mean of the intensity (I_O) of the three blanks was calculated. An outlier was omitted if one blank deviated more than 15 % from the mean, *i. e.* the mean relative standard deviation. For each individual test tube, the following quotient was calculated:

$$\Gamma = I_O/I_t - 1 \qquad 1$$

where I_t is the intensity of the soil sample, and log (Γ) was plotted against log (C):

$$\log(\Gamma) = a \times (\log C) + b \qquad 2$$

where C is the concentration of soil. A straight line was fitted to the curve and a preliminary EC_{50} calculated (*i. e.* the intersect with the log (Γ) = 0 line). From the preliminary EC_{50} the concentrations of the main test were calculated, where the highest concentration was 2.8 x EC_{50} (from $10^{(1.5 \times \log 2)} \times EC_{50}$) and a dilution factor of 2 was used between each concentration of the dilution series.

2.4.3 Main test in triplicate

2.4.3.1 Preparation of a dilution series of soil suspension

Fifteen 5 ml plastic test tubes (Microbics Corp.) were placed in a rack in a thermostate at 15 ± 0.1°C. To twelve of the tubes 1.5 ml of 2 % sodium chloride and to the three remaining tubes 3 ml soil suspension of the concentration obtained in the calculations under 2.4.2.6 above was added. The soil suspensions were serially diluted 1.5 ml to 1.5 ml 2 % sodium chloride for the three soil suspensions. Thus four concentrations of soil suspension with a dilution factor 2 in three replicates were obtained. The three remaining tubes contained 1.5 ml 2 % sodium chloride (blanks). All tubes were adjusted 10 min at 15°C.

2.4.3.2 Addition of bacteria

Twenty µl suspension of bacteria, prepared according to 2.4.2.2 above, was added to samples and blanks with 15 sec interval. The filter plungers were pressed through the tubes down to just above the surface of the suspension and the mixtures were shaken.

2.4.3.3 Incubation

The contact time between bacteria and soil particles was 30 min. Some times after the addition the mixtures were gently shaken, but during the last period (at least 5 min) the suspensions were allowed to settle to facilitate the separation of bacteria and soil particles.

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After 30 min the filter plunger was pressed through the tubes with 15 sec interval between each tube. A little more than the top two thirds of the suspension was pressed through the filter. The sedimented part of the incubated suspension was not filtered.

2.4.3.5 Light intensity measurements

One ml of each filtrate was transferred to cylindrical glass cuvettes and were temperature adjusted in the thermostate of the instrument. Five min after cessation of the incubation the light intensity of blanks and samples were read with 15 sec-intervals. Light intensity data were automatically recorded in our program. Calculations cf. 2.4.5.

2.4.4 Correction for light absorption and scattering

2.4.4.1 Sample preparation

Samples of each concentration after the measurement of light intensity in 2.4.3.5, (3 x 1 ml) were mixed in a glass cuvette and remaining bacterial activity was killed with 20 µl concentrated hydrochloric acid. The disappearance of light emission from the combined samples was checked in the instrument.

2.4.4.2 Preparation of bacterial suspension to the two-compartment cuvette.

One ml 2 % sodium chloride was temperature adjusted 10 min at 15°C. Fifty µl of bacterial suspension according to 2.4.2.2 above was added and the mixture was again thermostatted 15 min at 15°C.

2.4.4.3 Light intensity measurement of the blank, 2 % sodium chloride

The outer chamber of a two-compartment cuvette was filled with 2 % sodium chloride, thermostatted at 15 °C and the inner with the diluted bacterial suspension, prepared according to 2.4.4.2. Light intensity was read once every minute until a constant change in intensity was obtained (within 5-10 min). Usually a slow time-dependent decrease in the light intensity of the bacteria was observed.

2.4.4.4 Light intensity measurement of the sample with soil particles and killed bacteria.

Within one minute (*i. e.* between two measurements of the light intensity) the solution of the outer chamber was changed and replaced with sample with soil particles and killed bacteria. The measurement of light intensity was continued once a minute until a new process of constant change with time was attained. Usually this was obtained within 5-10 min. The time for the change of solutions of the outer chamber was noted.

2.4.4.5 The correction test of the three other concentrations of soil sample in the dilution series

The outer chamber was rinsed with two portions of temperature-adjusted (15°C) blank medium and filled with new blank medium. As in 2.4.4.3 the light intensity was measured with 1 min intervals till a constant change with time was observed. Then the solution of the outer chamber was changed to the second soil suspension according to 2.4.4.2 and 2.4.4.4.

Thus the light absorption and scattering of the four concentrations of soil suspension of the dilution series in the toxicity test were measured.

2.4.4.6 Calculation of correction of light absorption and scattering

Data was collected in a PC program written in Microsoft Quick Basic. Light intensity was plotted versus time in a diagram and linear regression was applied to the constant changes of the light intensities with time for blank medium on one hand and the soil suspension on the other (*cf.* above). The lines were extrapolated to the time point of change for the solutions in the outer chamber. The quotient between the calculated intensities of blank and sample were then used for correction of the intensity obtained in the main test (2.4.3.5). A data sheet for calculation of the light correction is shown in Appendix 1.

2.4.5 Calculation of a corrected EC_{50} for the sample and evaluation

A mean of the intensity (I_O) of the three blanks was calculated. An outlier was omitted if one blank deviated more than 15 % from the mean, i. e. the mean relative standard deviation. The intensities of the soil sample suspension were corrected for the contribution of light absorption and scattering according to 2.4.4.6. For each individual test tube, the following quotient was calculated:

$$\Gamma = I_O/I_t(\text{corr.})-1 \qquad 3$$

and log (Γ) was plotted against log (C):

$$\log(\Gamma) = a \times (\log C) + b \qquad 4$$

where C is the concentration of wet soil. Each replicate was treated separately and a straight line was fitted to each curve. Single outliers were omitted. EC_{50} was calculated (*i. e.* the intersect with the log (Γ) = 0 line) using least-squares fitting for each replicate.

A mean EC_{50} of the three replicates was calculated expressed in mg wet soil/ml and converted to dryweight. The reciprocal of the EC-value, here defined as a toxic unit (TU_{50}), expressed per g dry soil. Taking the content of organic matter (LOI) into account, the TU_{50} was also expressed per g organic material. A scheme of the calculations is shown in Appendix 2.

2.5 Microtox tests of toxicants in aqueous media.

Microtox tests of reference toxicants were performed according to the manufacturer's manual with some modifications (5,6). Tests were run in combined duplicates at pH 7.30 ± 0.05 with 5, 15 and 30 min exposure. 3-Methylbenzoic acid was a gift of Dr. A. Neilson at our institute.

2.6 Microtox tests of soil elutriates.

Water-based elutriates of soils were prepared and tested with luminescent bacteria. Soil (3 g) was mixed with 30 ml 2% sodium chloride and pH adjusted to 7.3 ± 0.1. The mixture was stirred for 1 h and then centrifuged at 15000 x g for 30 min. The supernatant was then tested according to the 100% procedure in combined triplicate, *cf.* under 2.5. Toxicity data of humous-coloured elutriates were corrected for light absorption according to a described procedure (13).

3. Result and Discussion

3.1 Test performance, dose dependence

The test strategy outlined for sediment tests was used for testing of soils. A preliminary EC value was obtained in a pretest covering a wide concentration interval. A main test with 4 concentrations close to the preliminarily determined EC value was then conducted. Both light scattering and light absorption interfered with the light measurements as shown in Fig. 1. The dose dependence of the uncorrected and corrected light intensity is shown in the figure. In most samples the corrections were within 10-20 % of the total intensity. In some samples, *e.g.* forest soil and the sample from, a high contribution from light absorption was observed, probably, due to a high content of humic substances. The necessity to correct for a considerable contribution of light absorption certainly reduced the precision of the test of the toxic effect.

The dose curve was used to calculate an EC_{50} expressed in mg soil/ml test medium. From this value a toxic unit (TU_{50} in (ml)/g) was derived by inversion of the EC_{50} and correction for the dry weight of wet soil.

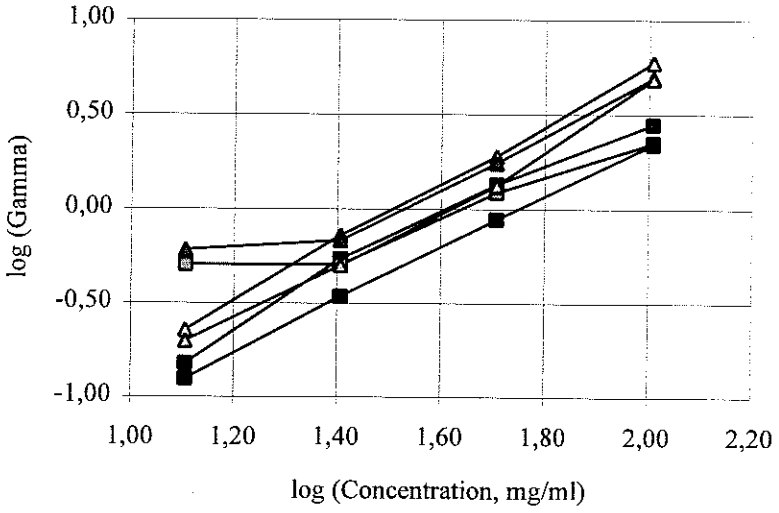

Figure 1. Dose dependence in a Microtox direct contact toxicity test of a soil, triangular symbols before and square symbols after correction for light scattering and absorption.

3.2 Reproducibility, variation of data.

EC_{50} values (mg dry soil/ml) were determined in triplicates from corrected dose curves and recalculated in toxic units (TU_{50}) as described above. In Table 1 the results of tests of separate replicates from each sample site is shown. Means and standard deviations were calculated. As a measure of the significance of the test technique, the mean of the relative standard deviations was calculated. Calculated from 19 tests the mean relative deviation was 15.0 %.

In the evaluation of test results weighted means of the replicates were calculated as follows: If a single replicate had a toxicity that was deviating more from the unweighted mean than 15 %, *i. e.* the mean of the relative standard deviations found for all samples, this value was considered an outlier and omitted, and a weighted mean of the remaining two replicates was calculated. When two values deviated more than this percentage, no omission was done. This way weighted means of the toxicities were obtained at the different sample sites, *cf.* Table 1.

Table 1. Microtox toxicity of soils.

Sample origin	Microtox toxicity (TU_{50}/g dryweight)** I	II	III	Mean (n=3)	Std dev (n=3)	Mean weighted	Elutriate (TU_{50}/g)	Organic content (%)	Chloro-phenols (µg/g)
Reference localities									
Artificial garden soil*	32.4	34.3	33.3	33.3	1.0	33.3	<<19	53.6	-
Garden soil, Uppland, summer	40.8	40.7	41.6	41.0	0.5	41.0	<<11	6.3	0.0067
Garden soil, Uppland, autumn	60.2	54.2	44.7	52.2	7.8	52.2	<<13	6.4	0.0182
Forest soil, Uppland, litter layer	598	510	670	586	80	586	52	89.3	-
Martiniemi, Finland, moraine, 1	≅6.1	≅4.8	≅4.4	≅5.0	≅0.9	<10	<<10	0.92	0.0125
Martiniemi, Finland, moraine, 2	12.1	10,8	12.4	11,7	0.9	11,7	<<11	1.4	-
Contaminated localities									
Haukipudas, Finland, silt, 1	505	372	576	474	104	541	330	2.0	648
Haukipudas, Finland, silt, 2	175	307	321	248	81	314	183	1.9	344
Oulainen, Finland, moraine	28.4	27.4	22.3	25.7	3.3	25.7	<12	5.2	117
Toras, Finland, wood debris	<56,5	≅181	≅207	≅107	≅80	≅194	≅14	67.2	1070
Spiked reference soils									
Garden soil:								6.3	
Phenol, x 1	107	110	123	113	8.7	113			
Phenol, x 2	147	100	97.0	111	28.1	98.7			
Phenol, x 4	227	237	181	212	29.8	212			
Garden soil:								6.3	
3-Methylbenzoic acid, x 1	87.6	82.8	86.7	85.6	2.5	85.6			
3-Methylbenzoic acid, x 4	119	109	120	116	5.9	116			
3-Methylbenzoic acid, x 10	191	167	209	187	20.8	187			
Salinity dependence									
Garden soil:								6.3	
2 % Sodium chloride	28.8	29.1	20.6	25.5	4.8	29.1			
3.1 % Sodium chloride	39.1	32.2	54.5	40.0	11.4	35.6			
4 % Sodium chloride	57.1	46.0	62.0	54.2	8.2	54.2			

* Composed of 70% peat, 25% pyroclastic rock (perlite) and 5% sand.

** TU_{50} (/g dry weight), toxic units as reciprocal EC_{50} in dry soil/ml at the specified test conditions.

3.3 Influence of salinity

The effects of variation of the salinity was studied in a series of tests. Suspensions of soil from a reference locality were prepared in media with different concentrations of sodium chloride. The results are shown in Table 1. A small increase in toxicity was indicated when increasing the salinity of the test medium. This is interpreted as more substances became available to exert toxic effects on the bacteria when the salinity increased. Although the effects were stronger in higher concentrations of sodium chloride, 2% concentration was chosen as test condition due to the number of reference compounds that has been tested as pure substances at this condition.

3.4 Reference localities

TU_{50} values from <10 to 586 per g dry soil were obtained from contaminated and uncontaminated localities (weighted means, Table 1). The lowest effects were found in garden soils, a sandy soil from a locality with no known contamination. From <10 to 50 TU/g could obviously be expected effect levels in a reference soil. The soil structure (and composition) influenced the test results. The forest soil with a high organic content had a strong effect in the test system, probably not related to anthropogenic contaminants. The suspension was dark coloured by humic substances that had to be corrected for in the light measurements, but apparently dissolving components of the soil, mainly composed of decaying spruce needles, also affected the bacteria. The observation points to the need of a careful interpretation of contaminated soils.

3.5 Contaminated soils

Soils from industrial sites previously used for wood preservation contained high amounts of 2,3,4,6-tetrachlorophenol and pentachlorophenol, *cf.* Table 1, and small amounts of other chlorinated substances (9-12). Two samples from Haukipudas taken in summer and autumn were toxic as tested in the direct exposure Microtox test. The summer sample contained higher amounts of the chlorophenols and was also more toxic than the autumn sample. The soils were treated in a soil remediation program and the lower concentration after the summer period was therefore expected.

Also soil from a remediation site at Oulainen contained chlorophenols, but the measured effect was unexpectedly low and close to the levels of the reference samples. This moraine soil was low in organic content as were the soils from Haukipudas. A soil from a saw-mill at Toras contained 1070 mg/kg dryweight of pentachlorophenol and 2,3,4,6-tetrachlorophenol. The soil had also an unexpectedly low effect in the test system, but this soil contained a high fraction of organic material. Low effects in soils with high contents of contaminants may reflect limited availability. In organogenic soils the

contaminants may be strongly bound to the soil matrix and as these soils have been subjected to microbial degradation, easily accessible fractions of the contaminants may have been converted. The remaining fraction of chorophenols could be tightly bound to the soil particles, organogenic and minerogenic, and thus inaccessible to the luminescent bacteria (14).

3.6 Spiked soils

Experiments were conducted to trace the toxicity from added known toxicants. In one experiment phenol, a common reference toxicant, was added to a reference garden soil at three different proportions. The toxicity of phenol in aqueous solution (EC_{50}) was determined at 30 min exposure as 25.5 mg/l. Mixtures of soil in 26, 51 and 99 mg phenol/l were tested in the solid phase test system and the results as TU_{50} are shown in Table 1. Phenol concentrations were analysed by gas chromatography after extraction of soil suspensions and derivatisation. The EC value of the reference soil decreased as expected upon addition of phenol (Table 1). However, the effect increase was not directly proportional to the phenol addition. Some of the phenol was not detected in analyses of the suspension, probably due to unavailability in the extraction procedure.

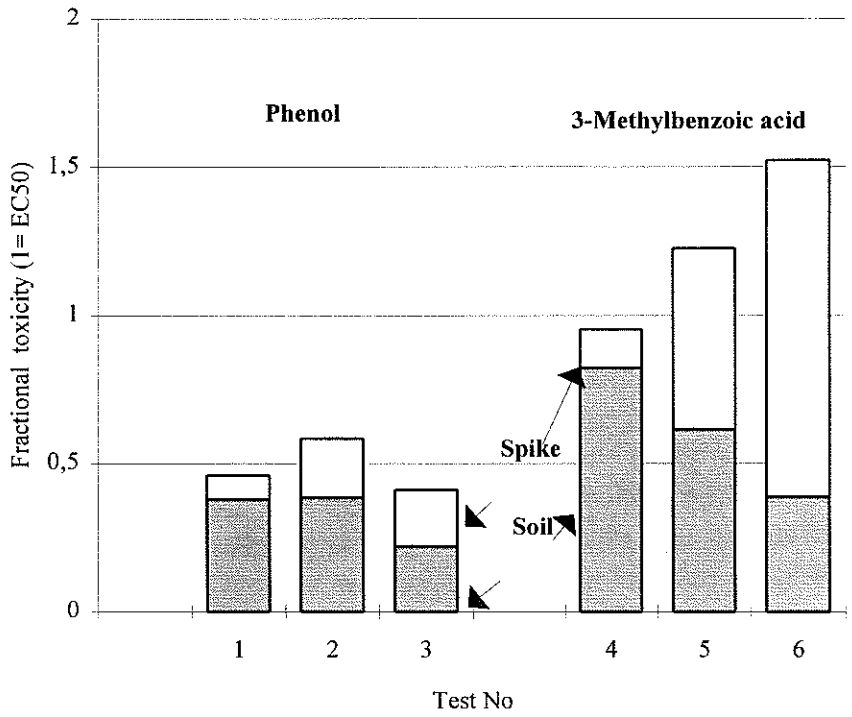

Figure 2. Contribution of soil and added toxicants to the Microtox toxicity tested in the direct contact test.

Calculating the fractional toxicity from both nominal added and analysed concentrations, the effects of phenol and the contribution from the uncontaminated soil could not account for the whole effect of the spiked soil. Only about half of the effect was explained by the amounts of soil and the added phenol. Co-operative effects were thus indicated.

A metabolite of xylene, 3-methylbenzoic acid was also added to soil at different proportions and then tested in the test system. The toxicity of 3-methylbenzoic acid in aqueous solution was determined as 129 mg/l (30 min EC_{50}). The additions of the substance corresponded to 137, 547 and 1367 mg/l. The test results with mixtures of a reference garden soil as TU_{50} are shown in Table 1. The toxicity increased in a dose-related way upon addition of the benzoic acid derivative. The relative response of soil and the toxicant in the mixture was calculated and the results are presented in Fig. 2. The soil intrinsic effect was decreasing and the contribution of the added methylbenzoic acid increased. However, the sum of the contributions was not constant in the three tests, but increased. The interpretation of this observation is not clear. There seemed to be a dose-dependent antagonistic effect when adding 3-methylbenzoic acid to the reference garden soil.

3.7 Comparison with tests of elutriates of soils

Results of tests of water-based elutriates of soils are presented in Table 1. The reference soils had no toxic effect as tested in the Microtox test system. In fact the garden soils stimulated the light production of the bacteria, an effect sometimes attributed to addition of some condition factors, like Ca and K (15). The calculated effect levels of the reference soils are, due to the normalisation per dryweight soil, heavily overestimating a real effect level. The forest soil with no known contamination was also toxic in the test as elutriate in accordance with the response in the direct exposure test.

Toxic effects were observed in the soil samples from Haukipudas, contaminated with chlorinated phenols. The toxicity was calculated as 330 and 183 TU/g dryweight, the former prior to and the latter after a 4 months treatment period for removal of chlorophenolic substances. The toxicity corresponded to about 60 % of the toxic effects determined in the direct contact test. Elutriates contain the more water soluble substances while the direct contact test may also assess particle-bound toxicants. The percentage of elutriate toxicity depends on the solubility of the main toxicants of a soil sample.

The chlorophenol contaminated soils from Oulainen and Toras, that had unexpectedly low toxicity in the direct contact test, had also very low effects tested as elutriates.

These observations support the interpretation of a strong influence by the bioavailability of the chlorophenolic substances.

4. Conclusions and comments

Light absorption and (to a lesser extent) light scattering of soil particles interfered with the intensity measurements in the Microtox toxicity test with direct contact between bacteria and soil. In this modified procedure the contribution of light absorption and scattering of soil particles is considered.

Technically soil samples may be tested in the proposed direct contact test. The procedure is somewhat more time-consuming than the original procedure (3). The mean of the relative standard deviations in the triplicates was 15%.

The interpretation of test results needs more basic data. The span between the highest detected soil toxicity and the detection limit was smaller than the corresponding span observed in contaminated sediments (7,8). The toxicity was higher than that obtained in water-based elutriates supporting the hypothesis that the direct exposure also assessed a fraction of particle-bound contaminants. There were obviously both false negative and false positive test results.

The dose-effect relations in spiked soil samples were complex. The importance of bioavailability is emphasised.

The modified Microtox Solid-Phase Test should preferably be applied tests, where relative responses are evaluated rather than absolute values. Such applications could be in solid waste management before and after remediation operations. Other applications could be in localisation of contaminated sites, when proper control soils with similar structure and composition could be obtained.

More research is required to explain the appearance of false negative and false positive responses and the deviation from proportionality in soils samples spiked with toxicants. Comparison with other tests of (acute) soil toxicity is also important future research.

5. Acknowledgements

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Appendix 1

Microtox 1996-02-13 MT2
IVL-Stockholm 07:28:46

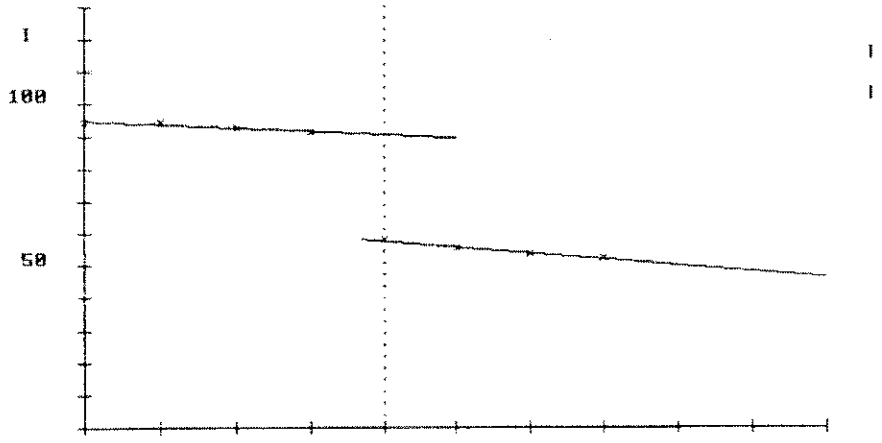
FÄRGKORREKTION

Prov: Oulainen 100/1 (JORDOU/1)

Testdatum: _____ Fil: .mtf Bakteriesats nr: M_____

Frysförvarat < > Filtrerat < > pH-justering från: _____
Kylförvarat < > Centrifugerat < > till: _____

tid	Avl	aktivp
---	----	----
0.0	94.4	1
1.0	94.3	1
2.0	92.7	1
3.0	91.3	1
3.5	0.0	0
3.6	0.0	0
4.0	provtillsats	
4.0	58.1	1
5.0	55.3	1
6.0	53.9	1
7.0	52.3	1
8.0	0.0	0
9.0	0.0	0
0.0	0.0	0



c= 100 I0= 90.38 Iff= 57.72

Calculation sheet, Microtox soil direct exposure test

Appendix 2

Sample locality: Oulainen, waste deposit site, N Finland
Test date: 1996-02-07

Soil Conc. (mg/ml)	Intensity (I)
Blank	
0,00	92,81
0,00	99,35
0,00	93,03
Mean	95,06

Mean EC_{50}	49,1
Mean TU/g dw	25,7
std dev	3,3
TU/g org mtrl	498

	Intensity	I(corr)	Gamma (I/I(corr)-1)	log Conc	log Gamma	slope	intercept	EC_{50} mg/ml	TU_{50} /g dw
Sample 1									
102,13	13,61	21,31	2,80	2,01	0,45	1,39	-2,30	44,5	28,4
51,07	32,78	34,52	1,34	1,71	0,13				
25,53	55,01	52,33	0,55	1,41	-0,26				
12,77	77,45	70,28	0,15	1,11	-0,82				
Sample 2									
102,13	15,98	25,02	2,23	2,01	0,35	1,07	-1,78	46,1	27,4
51,07	34,59	36,42	1,22	1,71	0,09				
25,53	56,39	53,64	0,51	1,41	-0,29				
12,77	59,06	53,59	0,51	1,11	-0,29				
Sample 3									
102,13	16,15	25,29	2,20	2,01	0,34	1,38	-2,42	56,7	22,3
51,07	40,83	42,99	0,88	1,71	-0,05				
25,53	63,28	60,19	0,34	1,41	-0,46				
12,77	79,27	71,93	0,12	1,11	-0,90				

Light absorption and scattering correction
Sample locality: Oulainen, waste deposit site, N Finland

Soil Conc. (mg/ml)	Io	Iff	Correction factor (Iff/Io)
102,13	90,38	57,72	0,64
51,07	86,13	81,80	0,95
25,53	79,71	83,80	1,05
12,77	80,07	88,24	1,10
0	80,89	95,04	1,17

Dryweights and loss of ignition

Tara	Wet-weight + tara	Dry-weight + tara	Glow residue + tara	Wet-weight	Dry-weight	Glow residue	Dry-weight (% of ww)	LOI (% of dw)
34,1834	53,3336	49,3439	48,5616	19,1502	15,1605	14,3782	79,2	5,2

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