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Relationship Between Contaminant Loss and Toxicity During Phytoremediation Using Solid-Phase Microtox® Tests

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In recent years, phytoremediation has been shown to be an effective means of lowering concentrations of metals and organics in contaminated soils and sediments (Anderson *et al.* 1993; Erickson *et al.* 1994). Although many phytoremediation studies have tracked contaminant concentration over time, the authors know of no studies that have determined relative toxicity reductions. A fast, inexpensive, and reliable toxicity test to adequately determine risk reduction in lieu of expensive chemical analyses would be beneficial when using phytoremediation as a method of choice in contaminated site cleanup.

In 1992, Azur Biologicals (formerly Microbics Corporation) introduced a solid phase toxicity test based on the popular Microtox® luminescence assay with *Vibrio fischeri.* Until that time, Microtox testing of soils and sediments had been performed on aqueous extracts of the media. Complications inherent in making whole-sediment/soil dilutions and the effect of contaminant accessibility and availability on particles, however, warranted a method that would expose bacteria to solid-phase media. The Microtox Solid-Phase Test (SPT) allows sample particles to come into direct contact with test organisms for a fixed period of time. Advantages of the test are the ability to rapidly detect both organic and inorganic toxic materials without sample extraction in a cost-effective manner (Kwan and Dutka 1992; Day *et al.* 1995). This test has been used as a screening assay and has shown positive correlation with invertebrate assays (Day *et al.* 1995). The Microtox SPT has proven to work well for single pure compounds, including PAHs (Tung *et al.* 1990; Azur Environmental 1995), but has not been validated for complex mixtures of soil contaminants, such as those found at industrial sites.

Use of the Microtox SPT was further investigated during a bench-scale phytoremediation study. The sensitivity of the Microtox SPT was assessed by monitoring changes in toxicity during phytoremediation of soils contaminated with relatively low concentrations of polynuclear aromatic hydrocarbons (PAHs) and metals. The objective of the study was to determine if, compared to untreated but contaminated controls, the toxicity of soils, as determined by Microtox SPTs, decreased as contaminant levels decreased with phytoremediation.

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MATERIALS AND METHODS

Soils used in the study were collected from a former manufactured gas plant site that operated from 1889 to 1974 in Newark, New Jersey. Sampling procedures and site characteristics are reported in IGT (1995). Two soils (A and B) from different locations at the site, predominantly contaminated with 5- and 6- ringed PAHs, were examined. Soil A contained PAHs with a mean concentration of 185 mg/kg total PAH (16 priority compounds ranging from 2- to 6-ringed structures) and was not pretreated prior to phytoremediation. Soil B was pretreated with a chemical/biological method to remediate PAH-contaminated soils (Pradhan *et al.* 1998). Pretreatment reduced total PAH concentration in Soil B from 1000 to 103 mg/kg. A third soil, Soil C, was contaminated with metals including chromium, copper, lead, nickel, and zinc that ranged from 14 to 230 mg/kg. Prior to beginning the experiments, all soil was passed through a 1 -mm screen while moist and stored at 4°C until used. Soils and plant tissues were analyzed for 16 PAHs ranging from 2 to 6-ringed structures according to USEPA SW-846 Method 3540 (Soxhlet extraction; USEPA 1992) using a 1:1 mixture of acetone and hexane for extraction. Total PAHs were quantified using USEPA SW-846 Method 8270B (USEPA 1992). This method was modified by incorporating a 30 m x 0.25 i.d. XTI (Restek) column, with an oven temperature profile of 40°C for 4 min, 40°C to 300°C at 10°C $min.^{-1}$; hold for 20 min. Metals were analyzed by USEPA Method 6010 (USEPA 1983). The percent of combustible solids and the moisture content of the three soils used in this study were determined prior to phytoremediation. Soil dry weight was determined after drying to constant weight at 90°C. After drying, the soil was combusted at 500°C for 5 hours and the combustible solids weight was determined by taking the difference between the ash weight and the dry weight. Particle size distribution was determined by hydrometer method (Sheldrick and Wang 1993). Soil pH was determined on the samples just prior to Microtox testing, following the manufacturer's protocol.

Phytoremediation experiments are described in Pradhan *et al.* (1998). Briefly, separate experiments that utilized Soils A, B, and C were carried out in duplicate in containers set up in the laboratory under artificial lighting at room temperature. Each experiment utilized 1) a treatment in which plants were grown, 2) a control soil that was manipulated in the same way as the treatment soils with no plants, and 3) an archived soil, designated t_0, that was not manipulated in any way but was stored in a sealed container at 4°C. Both the treatment soils and the control soils were fertilized weekly with Miracle-Gro™ diluted according to the manufacturer's directions. Contaminant concentrations in soils were analyzed at the beginning of the experiments. At the end of 180 days, samples of plant tissue and the soils from each pot were collected for contaminant analyses.

For the experiments that used PAH-contaminated soils (Soils A and B), 350 grams of soil were placed in each 4-inch wide x 2-inch long x 4-inch deep

container. Three separate plant species were selected and tested, according to their ability to remediate soil in previous studies (Pradhan *et al.* 1998): alfalfa (*Medicago sativa*), switchgrass (*Panicum virgatum*), and little bluestem (*Schizachyrium scoparium).* Seven grams of seeds from each species were planted in each container (one species per duplicate set of containers). For the experiment that used metal-contaminated soil, 1050 g of soil were placed in each 6-inch wide x 4-inch long x 9-inch deep container. Two plant species were tested: wheat (*Triticum aestivum*) and mustard (*Brassica juncea*). Twenty-one grams of seeds from each species were planted in each container (one species per duplicate set of containers).

Microtox SPTs were conducted on test and control soils at test completion and after 12 months of storage in sealed containers held in the dark at 4°C. In addition, Microtox SPTs were conducted on archived soils at the beginning of the study, after 180 days and 12 months of phytoremediation, using the same storage conditions as test soils. Assays were conducted according to standardized solid-phase protocols with the Microtox Model 500 analyzer (Azur Environmental 1995). All materials and reagents were purchased from Azur Environmental, Carlsbad, California, USA. Reconstituted cells of *V. fischeri* were exposed in duplicate to each of the duplicate soil samples. Exposure duration was 20 minutes at 15°C. All soil samples were osmotically adjusted with an aqueous NaCl solution (salinity = 3.5%). Soil suspensions were then filtered, and the bioluminescence of the supernatant was measured with the Model 500 analyzer according to the procedure. A log-linear model was used to calculate the EC_{50} (the contaminant concentration that reduces light production by 50% relative to water controls, expressed in "percent dry weight" of soil). EC_{50} was calculated using Microtox data reduction software for the SPT (Azur Environmental 1995). The relationship of EC_{50} results between archived soils and treatments after various intervals was tested using one-way ANOVA.

RESULTS AND DISCUSSION

Soil physical parameters for the three soils showed similarity in combustible solids, pH, and particle size distribution (Table 1). After 180 days, contaminant concentrations in each of the three experiments decreased. Total PAHs from Soil A decreased 47% to 57% from an original concentration of 185 mg/kg in archived soil (Tables 1 and 2). A decrease in total PAHs, although not statistically significant, was also seen in controls from Soil A (Pradhan *et al.* 1998). This indicates that processes (e.g., microbiological degradation) may have been active, in addition to phytoremedial processes such as plant uptake, transformation and/or rhizosphere-enhanced degradation, during the study. Similar decreases in contaminant concentrations were seen in phytoremediation treatments from Soil B, which had been amended by chemical/biological treatments. Total PAH concentration decreased by 8% to 15% from the original 100 mg/kg

Table 1. Chemical and physical characteristics of soil samples at beginning of study; values represent mean ± 1 SD of triplicate samples.

Parameter	Soil A	Soil B	Soil C
Total PAHs, mg/kg	184.5 ± 14.0	102.7 ± 4.7	<10
Cr, mg/kg	<10	<10	13.5 ± 0.5
Cu, mg/kg	<10	<10	50.5 ± 0.5
Pb, mg/kg	<10	<10	175.0 ± 55.0
Ni, mg/kg	<10	<10	17.0 ± 1.0
Zn, mg/kg	<10	<10	108.0 ± 2.0
Total Metals, mg/kg	<10	<10	364.7 ± 81.9
Combustible Solids, %	1.2 ± 0.1	0.8 ± 0.0	1.0 ± 0.1
Moisture, %	23 ± 2	4 ± 1	14 ± 7
Sand, %	87 ± 1	88 ± 0	83 ± 0
Silt, %	6 ± 0	7 ± 0	7 ± 0
Clay, %	7 ± 0	5± 0	10 ± 1
PH	7.20	6.57	7.84

Table 2. Mean contaminant concentrations in Soils A – C after phytoremediation. Data reprinted from Pradhan *et al.* (1998)

Soil	Contaminant	Treatment	mg/kg*	Mean % decrease after 180 days
A	Total PAHs	Control	135.9 ± 25.5	26.3
		Alfalfa	80.2 ± 8.9	56.5
		Switchgrass	79.5 ± 3.7	56.9
		Bluestem	97.1 ± 18.7	47.4
B	Total PAHs	Control	107.8 ± 6.3	None
		Alfalfa	86.9 ± 6.4	15.4
		Switchgrass	93.2 ± 4.7	9.3
		Bluestem	94.5 ± 1.1	8.0
C	Total metals	Control	318.1 ± 0.1	12.8
		Mustard	325.7 ± 74.1	10.7
		Wheat	278.2 ± 22.1	23.7

* Values represent mean values ± 1 SD of 3 replicates.

concentration (Tables 1 and 2). A reduction in metals was also seen in Soil C, where mean concentrations of total metals decreased by 11% to 24%. Analyses on plant tissues grown in Soil C showed metals that ranged from 5.8 mg/kg of chromium to 200 mg/kg of zinc. However, less than 10 mg/kg total PAHs were detected in any species grown in Soils A or B.

Microtox soil toxicity failed to show significant decreases with contaminant reductions in any of the soils tested after 180 days, using one-way ANOVA (Tables 3 and 4). Significant reductions in soil toxicity were not seen in metals-contaminated soil (Soil C) until 550 days (Table 4). Microtox EC_{50} values indicate that toxicity was not reduced by phytoremediation, but other intrinsic processes such as microbial activity over the course of the year's storage time after phytoremediation may have contributed to the significant reduction in toxicity seen in both treatments and controls from Soil C.

Table 3. Microtox® SPT EC_{50} values before and after phytoremediation for PAH-contaminated soils.

Number of days after phytoremediation	Treatment	Soil A EC_{50}[1]	Soil B EC_{50}
0	Archived	3.17 ± 1.32	1.61 ± 0.75
180	Control	4.08 ± 1.80	3.88 ± 0.77
180	Alfalfa	2.27 ± 0.75	5.99 ± 2.79
180	Switchgrass	2.42 ± 2.73	4.28 ± 3.39
180	Bluestem	1.90 ± 0.49	3.48 ± 0.85

[1] EC_{50}s are expressed as mean percent dry weight of soil ± 1 standard deviation, where n = 4.

Table 4. Microtox® SPT EC_{50} values measured at time zero, after phytoremediation, and one year after phytoremediation for metals-contaminated soil (Soil C).

Time (days)	EC_{50} values*			
	Archived	Control	Wheat	Mustard
0	0.99 ± 0.01			
180		2.51 ± 1.29	0.83±0.11	0.71 ± 0.32
550		2.80 ± 0.56[1]	2.37±:0.19[1,2]	2.11 ±0.16[1,2]

* EC_{50} values are expressed as mean percent dry weight of soil ± 1 standard deviation, where n= 2. Superscripts denote significant differences in EC_{50} values between time = 0 and time = 550 d (1), and time = 180 d and time = 550 d (2) where $p < 0.05$.

Previous remedial investigations reported that soils used in this study were contaminated predominantly with PAHs or metals (IGT 1995). The assumption was made that these contaminants were correctly identified as the chemicals of concern at the site of collection. Soil toxicity may be the result of compounds not monitored during this study. The chemical-biological treatment used on Soil B, however, likely did not contribute to toxicity, since previous Microtox SPT tests

performed in our lab indicate soil toxicity to decrease significantly after this treatment.

Soil toxicity demonstrated by the Microtox SPT has been shown to be dependent on soil composition. Clean soils with a high percentage of silt and clay show greater toxicity than those with a high percentage of sand (Ringwood *et al.* 1997). The soils used in this study were all characterized as sand or sandy loams, containing greater than 60% sand. The similarity of the three soils studied should have minimized any complications due to particle-size distribution effects on toxicity. Any changes in toxicity between pre- and post-phytoremediation are likely not due to factors previously experienced with the SPT (Greene *et al.* 1992; Benton *et al.* 1995; Ringwood *et al.* 1997).

Results obtained from this study indicate that the Microtox SPT may not be suitable for tracking relatively small but significant decreases in soil contaminant concentrations during phytoremediation. Confirmation of the minimal loss of toxicity during phytoremediation demonstrated in this study could be made using a battery of toxicity tests. Overall, these results indicate a need for including toxicity measurements along with analytical determinations during soil remedial activities. Further validation of the Microtox SPT with various soils differing in physical and chemical characteristics will determine whether this test is a useful screening assay for tracking toxicity changes following remediation.

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REFERENCES

Anderson TA, Gutherie EA, Walton BT (1993) Bioremediation in the rhizosphere. Environ Sci Technol 27:2630-2636

Azur Environmental (1995) Microtox® acute toxicity solid-phase test. Azur Environmental, Carlsbad, California

Benton MJ, Malott ML, Knight SS, Cooper CM, Benson WH (1995) Influence of sediment composition on apparent toxicity in a solid-phase test using bioluminscent bacteria. Environ Toxicol Chem 14:411-414

Day KE, Dutka BJ, Kwan KK, Batista N, Reynoldson TB Metcalfe-Smith JL (1995) Correlations between solid-phase microbial screening assays, whole-sediment toxicity tests with macroinvertebrates and *in situ* benthic community structure. J Great Lakes Res 21:192-206

Erickson LE, Banks MK, Davis LC, Schwab AP Muralidharan N, Reilly K. (1994) Using vegetation to enhance *in-situ* bioremediation. Environ Prog 13:226-231

Greene MW, Bulich AA, Underwood SR (1992) Measurement of soil and sediment toxicity to bioluminescent bacteria when in direct contact for a fixed time period. Proceedings of the Sixty-fifth Annual Conference and Exposition of the Water Environment Federation, New Orleans, Louisiana, 20-24 September, 1992

IGT (1995) Slurry-phase Bioremediation for Erie Street Site. Final Report Prepared by the Institute of Gas Technology, for Elizabethtown Gas Company Under Contract No. 40324

Kwan KK, Dutka BJ (1992) Evaluation of ToxiChromotest direct sediment toxicity testing procedure and Microtox® solid-phase testing procedure. Bull Environ Contamin Toxicol 49:656-662

Pradhan SP, Conrad, JR, Paterek, JR, Srivastava, VJ (1998) Potential of phytoremediation for treatment of PAHs in soil at MGP sites. J Soil Contam 7:000-000

Ringwood AH, DeLorenzo ME, Ross PE, Holland AF (1997) Interpretation of Microtox® solid-phase toxicity tests: The effects of sediment composition. Environ Toxicol Chem 16:1135-1140

Sheldrick BH, Wang C (1993) Particle size distribution. In: Carter MR (ed) Soil Sampling and Methods of Analysis. Lewis Publishers, Ann Arbor, MI, pp. 509-511

Tung KK, Scheibner G, Miller T, Bulich AA (1990) A new method for testing soil and sediment samples. In: Abstracts, 11th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Arlington, VA, p. 133

U.S. Environmental Protection Agency, USEPA (1983) Test methods for evaluating solid waste. Method SW-846-6010. Office of Solid Waste and Emergency Response, Washington, DC

U.S. Environmental Protection Agency, USEPA (1992) Test methods for evaluating solid waste. Method SW-846-8270B. Office of Solid Waste and Emergency Response, Washington, DC