Comparison of the Phytotoxkit Microbiotest and Chemical Variables for Toxicity Evaluation of Sediments

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ABSTRACT: The main objective of the research was to evaluate the suitability of the Phytotoxkit microbiotest as a tool for hazard assessment of sediments. The concentrations of oil derivatives, polycyclic aromatic hydrocarbons (PAHs), and heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn) were determined in sediment samples collected from the urban canal in Opole (Poland), in order to obtain a general insight of the level of sediment contamination. Phytotoxicity of sediments was estimated on the basis of seed germination and root elongation measurements, combined into an overall germination index (GI). The results revealed spatial and vertical differentiation in sediment contamination. A good correlation was obtained between organic matter content and the concentrations of particular sediment pollutants. Values of correlation coefficients at $P < 0.05$ ranged from 0.3246 for oil derivatives to 0.8929 for PAHs. Phytotoxicity tests, carried out on the monocotyl *Sorghum saccharatum* and the dicotyls *Sinapis alba* and *Lepidium sativum*, showed different responses of the three plant species to sediment samples ranging from growth inhibition to growth stimulation. The GI values revealed the following increasing order of plant sensitivity to contaminated sediments: *L. sativum* $<$ *S. alba* $<$ *S. saccharatum*. The study demonstrated that the Phytotoxkit microbiotest was effective in identifying toxic samples. However, sediment organic matter content and grain-size distribution had a significant impact on both sediment contamination and higher plant responses to contaminated samples. The implication of these findings are discussed. © 2006 Wiley Periodicals, Inc. Environ Toxicol 21: 367–372, 2006.

Keywords: sediments; contaminants; phytotoxicity; higher plants; microbiotest; toxkits; urban canal

INTRODUCTION

Microbiotests have been successfully used as a suitable tool for low-cost routine toxicity evaluation of contaminated aquatic environments (Blaise, 1991; Persoone and Vangheluwe, 2000). However, most of these tests are performed on liquid samples using organisms, such as bacteria, microalgae, and crustaceans, from different trophic levels. The toxicity associated with sediments has been measured mainly on aqueous elutriates, interstitial water (Cheung et al., 1997), and saline or organic extracts that can be fractionated (Carr et al., 1996). While these techniques have yielded useful data, they do not expose test organisms directly to sediment particles. Owing to this, the contaminant molecules bound to sediment particles are not in contact with the organisms. As soils and sediments may differ in their chemical, physical, and biological characteristics from one site to another, direct contact bioassays with test species of different trophic levels are also required for the adequate soil or sediment toxicity evaluation (Benton et al., 1995; Ross et al., 2000; Chial and Persoone, 2002). To meet such demands for the primary producers, a phytotoxicity microbiotest has been recently developed with three higher plant species: the monocotyl sorgho *Sorghum saccharatum* and the dicotyls mustard *Sinapis alba* and garden cress *Lepidium sativum* (Phytotoxkit, 2004).

Although higher plants are an essential part of a healthy and balanced ecosystem, the use of vascular plants to assess

environmental risk has only gained attention in recent decades. Most of the current standardized phytotoxicity test methods have been developed since the 1960s (Lewis, 1995). Higher plant phytotoxicity bioassays are based on seed germination, root elongation, and early seedling growth measurements. Various higher plant bioassays have been used to evaluate the phytotoxic effects of commercial chemicals (Günther and Pestemer, 1990), industrial and municipal effluents, hazardous wastes, leachates (Wang and Williams, 1988; Vasseur et al., 1998; Filidei et al., 2003), and contaminated soils or sediments (Olajire et al., 2005). So far, phytotoxicity tests have been conducted mainly to meet the regulatory requirements for commercial chemicals (primarily pesticides), as well as to monitor the ecotoxicity of contaminated soils during bioremediation processes. However, the use of these tests is slowly increasing due to the ecological importance of plants, increasing awareness of their sensitivity, and the increasing number of environmental regulations requiring their use. Recent reports have considered phytotoxicity tests to be useful in assessing sediment toxicity, particularly in terms of dredging activity and possible land application of the dredged material (Woodard, 1999; Chen et al., 2002).

The objective of the present study was (1) to characterize the surficial and vertical distribution of heavy metal, PAHs, and oil derivative concentrations in urban canal sediments; (2) to assess the toxicity of sediments through comparison of chemical and phytotoxicological analyses; and (3) to evaluate the potential of the Phytotoxkit microbiotest for sediment toxicity assessment.

STUDY AREA AND SAMPLING

The hydrographic sector selected for the study was the Młynówka canal, one of the main urban canals in the town of Opole in southern Poland, with a length of 1500 m, a mean width of 10 m, and a depth of 1.5 m. The Młynówka canal, situated in the central part of the town, is polluted by urban storm–water runoff and effluents from the welding equipment works. Two transects along the canal were assigned at ∼500 m from each other. Three sampling sites, which differed in respect of water level and time of exposure of the banks, were selected for each transect. Sediment samples from six sites were collected in January 2005, prior to the dredging works, when the lowest water level during winter season caused the sediment to be air-exposed at both bank-side sites (1 and 4). Sediment samples were collected with an Eckman-Brige grab (225 cm^2) and a core sampler (Ø 5 cm). Sediment cores, except from sites 3 and 6 situated in the middle part of the canal, were divided into 5 cm and 20 cm layers: A (0–5 cm), B (5–25 cm), and C (25–45 cm). The sediments were put in polyethylene containers and transported to the laboratory.

MATERIALS AND METHODS

Sediments were dried at room temperature, crushed, sieved over a 2 mm mesh sieve, and homogenized.

Chemical Analyses

The following physical–chemical parameters were analyzed using standard methods: granulometric composition, pH, electrical conductivity (EC), organic matter content, polycyclic aromatic hydrocarbons (PAHs), oil derivatives, and heavy metals (Cd, Cr, Cu, Hg, Ni, Pb, and Zn). Grain-size was assessed quantitatively according to Casagrande's aerometric method. Measurements of pH and EC were performed with a glass electrode on a sediment sample mixed with deionized water in a ratio of 1:2.5. Organic matter content was determined gravimetrically after combustion of 3 g of dried subsamples at 500°C. PAHs were analyzed by gas chromatography (VARIAN GC-3800), using a flame-ionization detector (GC-FID) and a 30 m × 0.25 mm ZB-5 column with a film thickness of 0.25 μm. Prior to GC analysis, sediment samples were extracted with a mixture of hexane and dichloromethane (60:40 v/v). The 16 studied parent PAHs ranged from di-aromatics (naphthalene) to hexa-aromatics (indeno [1,2,3-cd] pyrene). Oil derivatives were determined gravimetrically after previous ether extraction. For metal analysis, the samples were digested with a mixture of HNO_3–$HClO_4$ in a microwave oven. Heavy metal content was measured by atomic absorption spectroscopy (AAS) using a double-beam Philips UNICAM PU9100X spectrophotometer with deuterium background correction and mercury analyzer AMA 254.

Analyses of correlation coefficients between the physical–chemical parameters of sediments were conducted using Pearson Product Moment Correlation Coefficient. All results reported are the means of three replicates.

Toxicological Analyses

Sediment toxicity was assessed with the standard Phytotoxkit microbiotest, a commercial toxicity bioassay based on three species of higher plants: the monocotyl sorgho *S. saccharatum* and the dicotyls garden cress *L. sativum* and mustard *S. alba*. This microbiotest measures both the decrease/absence of seed germination and the decrease of root growth after 3 days of exposure of the seeds to toxicants or to contaminated soils/sediments. The bioassays were carried out in three replicates with each of the three test plants. For a comprehensive interpretation of the data, seed germination and root elongation (the two end-points of the Phytotoxkit microbiotest) were combined in a germination index (GI), according to the equation: GI = $(G_s L_s)/(G_c L_c)$, where G_s and L_s are seed germination (%) and root elongation

TABLE I. Basic properties of sediment samples from the urban canal of Młynówka (I transect)

| Sample | Grain Size (%) | | | pH | Conductivity (mS cm^{-1}) | Organic Matter (%) |
	Sand	Silt	Clay			
1A	34	60	6	6.95	0.442	8.85
1B	32	61	7	7.12	0.937	25.4
1C	35	38	7	7.5	0.492	29.0
2A	65	31	4	6.8	0.797	8.8
2B	93	6	1	6.8	0.448	1.9
2C	58	36	6	6.7	1.274	9.9
3	71	25	4	6.4	1.289	6.4

(mm) for the sample and G_c and L_c the corresponding control values.

RESULTS AND DISCUSSION

Physical–Chemical Properties of Sediments

Granulometric composition, pH values, electrical conductivity, and organic matter content of dried sediments are shown in Table I. The results of particle size analysis revealed that the 14 sediment samples were not homogenous physically. Samples collected from the bank section of the canal had a similar ratio between the granulometric fractions sand, silt, and clay. The silt fraction made up the majority of sediment particles in all layers (38–61%) at sites 1 and 4. Sediments collected from the other sampling sites were composed mainly of sand (58–93%), but at sites 2 and 5, they were more heterogeneous in relation to the depth of the sediment profiles. There was variation in pH values, which ranged from 6.4 to 7.5. Values of EC ranged between 0.448 and 1.289 mS cm^{-1}, indicating low and medium levels of sediment salinity, the latter especially in the middle section of the canal.

The analysis of organic matter content revealed a small spatial differentiation within the surface layers of sediments (0–5 cm), which contained from 6.4 to 8.85% organic mat-ter. However, the amount varied significantly below the depth of 5 cm. In comparison with surface layers, organic matter content increased with sediment depth to 25.4–29.0% at both bank-side sites 1 and 4. At sites 2 and 5, the sandiest middle layer of sediments (5–25 cm) in turn contained 1.9% organic matter.

Contamination of surface layers by oil derivatives and PAHs showed a similar spatial variability throughout the canal (Table II). The amount of these pollutants increased from the bank-line of the canal toward the middle. The content of oil derivatives increased from 0.56 g kg^{-1} d.m. in sample 1A to 3.2 g kg^{-1} d.m. in sample 3. The increase in the concentrations of oil derivatives with sediment depth was also noted, with exception of the sandiest layers B at sites 2 and 5. The total concentration of 16 PAHs varied from 3.2 to 12.7 mg kg^{-1} d.m. The lowest PAHs concentrations were detected at sites 2 and 5 (layers B). On the other hand, the highest levels of PAHs (9.5–12.7 mg kg^{-1} d.m.) were recorded at sites 1 and 4, in the subsurface layers. This difference in PAH contamination levels can be partly explained by sediment grain-size distribution and organic matter content, which ranged from 1.9 to 29.0%. Several authors have observed that PAHs are mainly associated with the organic matter due to their hydrophobic character, and fine grain-size sediments accumulate PAHs at greater concentrations than coarse sands (Baumard et al., 1999). The study of the parent PAHs according to their molecular

TABLE II. Oil derivative, PAHs, and heavy metal content in sediments of the urban canal

| Sample | Oil Derivatives (g kg^{-1}) | PAHs | | | Heavy Metal Content (mg kg^{-1}) | | | | | | |
		Total (mg kg^{-1})	%LMW	%HMW	Cd	Cr	Cu	Hg	Ni	Pb	Zn
1A	0.56	6.7	15.5	84.5	3.75	31.3	55.1	0.52	41.1	48.7	640
1B	1.83	9.5	6.4	93.7	3.9	88.3	96.5	1.06	49.8	92.2	1247
1C	2.06	12.7	16.6	83.4	3.88	127.2	89.3	0.83	43.8	97.2	1101
2A	1.11	6.9	23.0	77.0	2.61	32.6	48.7	0.36	37.0	41.3	588
2B	0.69	3.2	17.1	82.9	0.96	9.1	17.8	0.09	13.3	29.6	278
2C	1.99	6.6	13.9	86.1	3.86	37.9	77.2	0.82	43.1	58.9	1610
3	3.21	8.1	20.3	79.7	3.90	88.3	96.5	0.52	49.8	92.2	1247

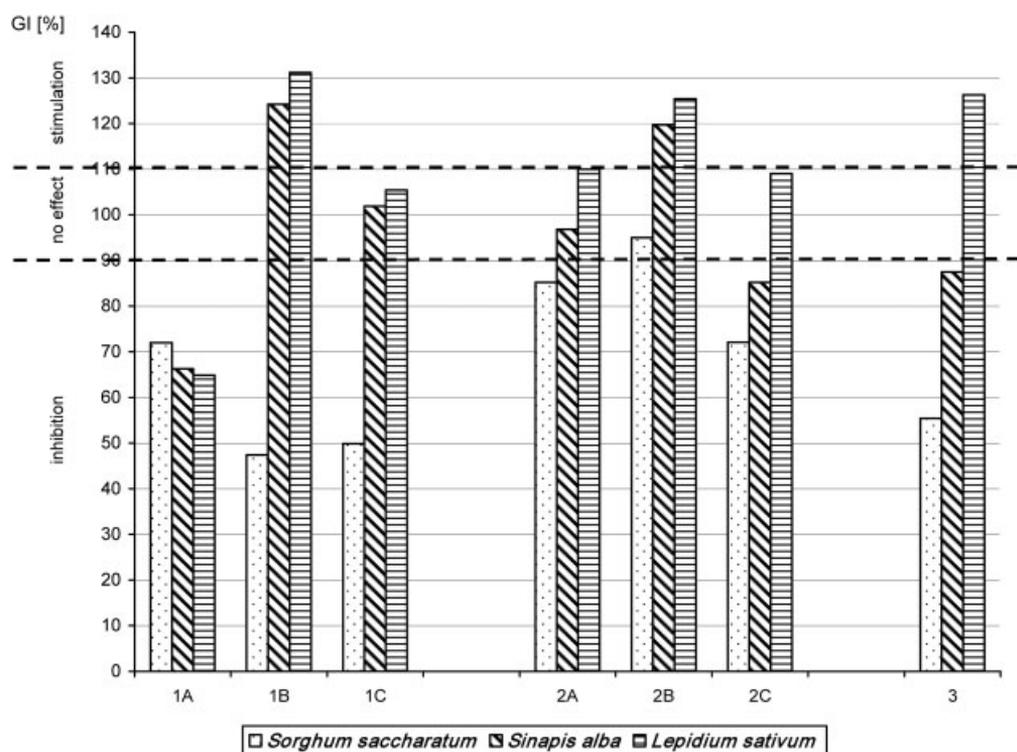LMW, Low molecular weight; HMW, high molecular weight.

Fig. 1. Germination index values (% of control) for sediment samples collected from the first transect.

weight can provide information on the process from which these compounds were generated. All analyzed sediments were contaminated mainly by high-molecular weight compounds, which formed from 77.0 to 93.7% of the total PAHs. This indicates their pyrolytic origin, though a contribution of petrogenic sources cannot be excluded (Baumard et al., 1998). Assessment of the potential toxicity or bioavailability of PAH residues is not simple, although according to Rogers (2002) the low-molecular weight PAHs are more likely to be released at significant concentrations, following resuspension of sediments into the water column, in comparison with the higher molecular weight ones.

A similar pattern of sediment contamination was noted for heavy metals. Trace metal concentrations (Cr, Cu, Pb, and Zn) were ~2–3 times higher in the middle part of the canal, compared with those in the bank-side. When comparing heavy metal concentrations in the sediment profiles, it is clear that their amount increased with the depth of sediments, except Ni content, which remained rather constant. Heavy metal uptake by sediments is clearly related to the content of organic matter (Lin and Chen, 1998) and the granulometric composition (Soares et al., 1999). However, metal increase, especially Cr, Hg, Pb, and Zn, varied in respect of sediment granulometry. The increase of the average Cr and Pb concentrations was four and two times higher, respectively, in silty sediments (sites 1 and 4), whereas in sandy ones (sites 2 and 5) it was only nearly half of these values. In contrast, the average Hg and Zn content

revealed a similar slightly higher increase in sandy sediments (2.3 and 2.7 times, respectively) in comparison with silty ones (1.7 times). There was a high correlation between the concentrations of particular heavy metals. Values of correlation coefficients ranged from 0.6155 ($P = 0.001$) between Cr and Zn to 0.9463 ($P = 0.001$) between Cd and Ni, respectively. The results of correlation analyses confirmed that heavy metal concentrations were significantly ($P = 0.001$) correlated with organic matter content. The correlation coefficients, which ranged between 0.3612 and 0.8167, increased in the order Zn < Ni < Cd < Pb < Cu < Cr < Hg. For most of these trace metals, a similar mobility order has been observed by Guevara-Riba et al. (2004). Organic matter content was also correlated with the levels of other contaminants in the sediments. Correlation coefficients for PAHs and oil derivatives were 0.8929 ($P = 0.001$) and 0.3612 ($P = 0.001$), respectively.

Sediment Phytotoxicity

Among different toxicity indices based on germination and seedling growth of various higher plants, the GI seemed to be a good method for soil and sediment toxicity evaluation (Beltrami et al., 1999; Mishra and Choudhuri, 1999). In the present study, the three plant species of the Phytotoxkit microbiotest responded differently to the degree of contamination of the sediment samples (Fig. 1). In general, GI values clearly

revealed the inhibitory effects of sediment contaminants on seed germination and root elongation of sorgho *S. saccharatum*. The majority of GI values decreased when comparing surface (A) and lower (B and C) layers of sediments, as well as the bank and the middle sections of the canal. These results were consistent with chemical data as GI decrease corresponded to higher concentrations of heavy metals, PAHs, and oil derivatives. For *S. alba* and *L. sativum* in turn GI values showed diversified effects, which ranged from growth inhibition to growth stimulation. With higher pollutant concentrations, the decrease in GI figures became more pronounced with *S. alba* than with *L. sativum*, indicating that the former species was relatively more sensitive to the toxic effect of contaminated sediments. However, and unexpected, for both species, the germination indices for more polluted lower layers of the bank-side sites either were not statistically different from the controls or showed a significant stimulation. This suggests that there are other factors, not measured in this study, that contribute to the phytotoxicity of the analyzed sediments, such as chemical forms of heavy metals, which determine their mobilization capacity and bioavailability (Morillo et al., 2002) or sediment nutrient status (Nakamura et al., 2002). Higher amount of nutrients adsorbed on organic matter-rich sediment layers might conceal the inhibitory impact of phytotoxic contaminants in sediments and hence give a false negative assessment as reported by Gong et al. (2001). Additionally, interactions between contaminants may result in antagonistic or synergistic effects that are difficult to predict (Kabata-Pendias and Pendias, 1999). Fargašova (2001) reported that Cu had a strong antagonistic effect to Ni and reduced its unfavorable effects. Consequently, combination of these metals significantly stimulated root growth of mustard *S. alba* seedlings in comparison with the control. However, what kind of interactions will appear depends on both the analyzed parameters and the plant species used (Dukhovskis et al., 2003). Our phytotoxicity data confirm that the range of tolerance to environmental factors is an important characteristic, which reflects the effects of anthropogenic stress factors on living organisms.

In conclusion, the data of this study revealed that the Phytotoxkit microbiotest was effective in identifying toxic samples, although the three plant species responded differently to the sediment samples. The values of the germination indices based on seed germination and root elongation bioassays revealed an increasing plant sensitivity to the contaminated sediments in the following order: *L. sativum* < *S. alba* < *S. saccharatum*. Moreover, a wide range of GI values indicates that the response of test plants to sediment contaminants (heavy metals, PAHs, and oil derivatives) depended not only on pollutant concentrations but also on sediment characteristics. In terms of phytotoxicity, sediment organic matter content and grain-size distribution had a significant impact on plant responses. Despite the higher contaminant concentrations, sediment samples that were rich in organic matter and composed mainly of silt caused the highest adverse plant response. They indeed inhibited both seed germination and root elongation of *S. saccharatum* significantly and on the other hand stimulated root growth of *S. alba* and *L. sativum*. In this context, further studies should be performed on sediment characteristics for a better understanding of the biological/ecotoxicological response to the contaminants present.

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