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Development of Reference Sediment Samples for Solid Phase Toxicity Screening Tests

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Toxicity and genotoxicity screening of sediments, suspended sediments, suspended particulates, soils, solid wastes and other solid phase samples have provided monitoring agencies many difficulties and challenges over the years. Initially very few solid phase bioassays were performed because of technical difficulties. These bioassays were usually carried out by using higher organisms (earthworms, benthic invertebrates such as chironomids, mayflies, amphipods and fresh water oligochaetes) and seeds or plants which are normally found in soil and sediment samples, for example (Day et al., 1995). As microbial and enzyme based bioassays were developed or their potentials realized, solid phase extracts (water and solvent) were used to assess the toxicity of these solid phase samples. However, in the routine toxicity screening of solid phase samples, it was often difficult to selectively detect the presence of toxicants due to their low concentration, low solubility or insolubility in the extracting solvents (Atkinson et al., 1985; Schiewe et al., 1985; Kwan, 1992).

To address this conundrum laboratories resort to a variety of extracting solvents and concentration procedures. The use of solvent extraction on solid phase samples has often been very chemical specific and there is the question to what degree the samples have been changed during the extraction process. Also it has been noted that although sediments may contain high concentrations of toxic chemicals, toxicity or increased toxicity to organisms living in the sediments may not be observed (True and Hayward, 1990). The bioavailability of toxic compounds to benthic organisms depends on the trophic position of an organism and any toxic effect to the organism depends on its relative sensitivity to interstitial and particle bound chemicals (Swartz et al., 1986). Extraction/concentration procedures do impact on the original bioavailability of the chemicals in the sample and in doing so the potential toxicity can be estimated but the real in *situ* toxicity is rarely known. Therefore, success in detecting the true toxicity of solid phase samples is still very limited.

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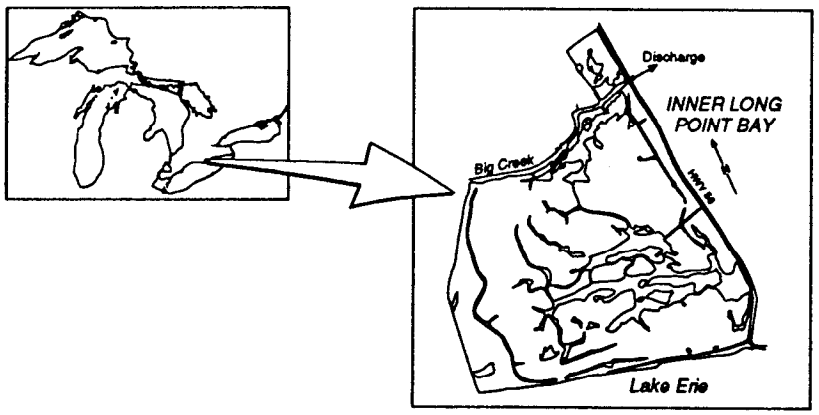
Over the past five or six years there has been a partial solution to the above problems. Qualitative and semi-quantitative direct solid phase toxicity testing procedures have been developed using bacterial systems (Dutka and Gorrie, 1989, Brouwer et al., 1990; Kwan, 1991) and using the immunochemical detection of a cell surface reporter protein of *Escherichia coli* (Stubner et al., 1994).

Direct solid phase toxicity testing has one major problem, the lack of standardized controls. Tung et al. 1990 and 1991 and Day et al., 1994 have used synthetic solid phase control samples prepared from silt, sand and clay for their blank controls. However, there are concerns that the makeup of the synthetic samples are physically and chemically different from natural sediment. Therefore we have tried to address this problem and concerns by preparing two standardized natural reference sediments, a non-toxic sediment and a toxic sediment. Details on the development of these two reference sediments and their responses to a direct sediment bioassay are given in this report.

METHODS AND MATERIALS

Sediments were collected from Big Creek marsh, Long Point, Lake Erie, Ontario (Fig. 1) from an area designated as a Canadian Wildlife Bird Sanctury. Details of sample collection procedures are described in Reynoldson et al. (1995).

Figure 1. Big Creek marsh sampling site, Lake Erie, Ontario



In the laboratory the sediments were pooled together into a large container and mixed thoroughly. The pooled sediments were sieved through a 250 µm mesh and washed with a constant flow of tap water. Washing and sieving was continued until a negative response was obtained in the monitoring bioassay (Toxi-Chromotest). After the final washing the sediment slurry was collected in a large sterile plastic container and was allowed to settle for 48 hours. After 48 hour settling, the overlying water was discarded by siphoning.

The toxicity of the sediments was checked by the Toxi-Chromotest kit following the DSTTP procedure developed by Kwan (1993) with some modifications, according to the manufacturer's instructions (Environmental Bio-detection Products Incorporation, 14 Abacus Road, Brampton, Ontario, Canada, L6T 5B7).

After the treated sediment was confirmed to be non-toxic, the sediment was divided into 500 g portions and freeze dried using the Lyph-Lock® Stoppering Tray Dryer Freeze Dry System (Model 77560). After the freeze drying process, the original 500 g portions were pooled into a large sterile plastic drum and rotated over a rolling machine for 48 hours to obtain a thorough mixing.

The wet untreated (natural) and the non-toxic sediments were submitted to the National Laboratory For Environmental Testing at CCIW for chemical analysis. The chemical composition of these samples is shown in Table 1.

Table 1. Chemical composition of untreated and treated sediment samples collected from Big Creek, Long Point, Lake Erie, Ontario

METALS	Untreated µg/g	Treated µg/g
Aluminium	0.94	0.83
Antimony	12.00	11.00
Arsenic	<5.00	<5.00
Barium	73.00	62.00
Cadmium	1.00	<0.20
Chromium	17.00	15.00
Copper	16.00	14.00
Iron	2.82	2.61
Lead	24.00	20.00
Magnesium	1.04	1.03
Manganese	616.00	516.00
Potassium	0.16	0.13
Sodium	0.09	0.09
Strontium	130.00	117.00
Tin	<20.00	<20.00
Titanium	0.30	0.32
Vanadium	18.00	15.00
Zinc	73.00	62.00

500 g of the freeze-dried non-toxic sediment was spiked with sufficient mercuric chloride to obtain a final concentration of 500 ppm. The sample was mixed

thoroughly by hand and then tested for toxicity using the DSTTP procedure and the Toxi-Chromotest kit. Table 2 presents the data obtained from this positive (toxic) reference sediment tested at 0, 3, 6 and 18 weeks after preparation.

Table 2. Toxicity data obtained from positive control sediment using DSTTP and Toxi-Chromotest kit.

Trials	Dilutions of spiked sediment*																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
A_2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
A_3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
A_4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
B_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
B_2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
B_3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
B_4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
C_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
C_2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
C_3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
C_4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
D_1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
D_2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
D_3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
D_4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+

- = 100% blue colour inhibition (toxic)
+ = Blue colour developed (non-toxic)
A = 0 week
B = 3 weeks
C = 6 weeks
D = 18 weeks

* 1: 50% (250.000 ppm)
2: 25% (125.000 ppm)
3 : 12.5% (62.500 ppm)
4 : 6.26% (31.250 ppm)
5 : 3.13% (15.625 ppm)
6 : 1.57% (7.813 ppm)
7 : 0.78% (3.906 ppm)
8 : 0.39% (1.953 ppm)
9 : 0.20% (0.977 ppm)
10 : 0.10% (0.488 ppm)
11 : 0.05% (0.244 ppm)
12 : 0.03% (0.122 ppm)
13 : 0.02% (0.061 ppm)
14 : 0.01% (0.031 ppm)
15: 0.005% (0.015 ppm)
16: 0.0025% (0.008ppm)
17 : 0.0013% (0.004 ppm)
18 : 0.0007% (0.002 ppm)

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of selected chemicals in the natural sediment collected from the Canadian Bird Century, Big Creek, Long Point, Lake Erie, Ontario, Canada, before and after washing and sieving through a 250 µm mesh.

The chemical concentrations in the treated sample were, overall, slightly lower than the untreated (natural) sample, with the exception of arsenic, sodium and tin which maintained their original concentrations. Titanium was 0.02 µg/g higher in the untreated sample. The implication of these observations is that the majority of these chemicals are firmly bound to the sediment particles and are not water soluble. The slight difference between the washed and untreated samples may be due to the washing out of water soluble organic and inorganic complexes which were not tested for and/or the loss of chemicals adsorbed to the particles which failed to pass through the 250 µm mesh sieve.

This similarity of chemical composition was also reflected in the DSTTP test results. The natural sediment before washing was found to be slightly toxic (2+) at the 50% concentration level, the most concentrated level which can be tested by this bioassay. Nine aliquots of the washed reference sample were found to be negative in this bioassay.

Environmental sediments are unstable if kept at room or low temperatures for long periods of time due to the continuous process of biodegradation activities within the sediment. Biodegradation activities within the sediment can result in altering the chemical level(s) and/or the chemical composition in the sediment and subsequently can produce inconsistent results. To minimize the biodegradation activities in the sediment and without changing the integrity of the sample, we freeze dried our washed and sieved sediment using the Lyph-Lock® Stoppering Tray Dryer, Freeze Dry System (Model 77560).

The freeze-dried sediment was then tested for toxicity using the DSTTP procedure. Result obtained from the freeze-dried sediment indicated that the sample was slightly toxic (50% concentration). This was not surprising. This toxic effect seen in the freeze-dried non-toxic reference sample was, we believe, due to the concentration of chemicals as a result of the removal of water (approximately 75% wet weight). Thus the chemical concentrations were increased approximately three fold.

In a routine DSTTP bioassay a 50% dilution (1:2 ratio of wet sediment to reaction mixture) is usually prepared for the first concentration. However, at this dilution it was very difficult to prepare a sediment slurry due to the freeze-dried sediment absorbing too much liquid. As a result, a 15 ratio (w:v) of freeze dried sediment and reaction mixture was used for the toxicity test. This non-toxic sediment, used in a 1:5 ratio was our non-toxic reference sediment.

The positive (toxic) reference sediment was evaluated for toxicity in quadruplicate using the DSTTP procedure and the Toxi-Chromotest kit immediately after preparation ($A_{1,2,3,4}$), three weeks after preparation ($Q_{1,2,3,4}$), six weeks after preparation ($C_{1,2,3,4}$) and eighteen weeks after preparation ($D_{1,2,3,4}$). The non-toxic freeze-dried reference sediment (1:5 ratio) was used as the negative control. Table

2 shows the results of these assays.

Toxicity was detected at the mean value of 0.039 ppm Hg^{++} concentration levels both at 0 and three weeks and at 0.031 ppm after six and eighteen weeks of storage. This demonstrates that the freeze-dried positive (toxic) reference sediment was stable for a mininum of eighteen weeks.

A non-toxic freeze-dried reference sediment can be prepared by collecting a natural environmental sample from a relatively pristine area and washing with running tap water and sieving through a 250 µm mesh. This non-toxic freeze-dried reference sample produces reproducible negative (non-toxic) responses when tested at the ratio of 1:5 (sediment: Toxi-Chromotest reaction mixture) and produces reproducible data. A 1:5 dilution was the most suitable freeze-dried sediment concentration to use for negative control with the DSTTP and the Toxi-Chromotest kit. The non-toxic reference sediment is stable at room temperature. A positive (toxic) reference sediment was developed by spiking the non-toxic freeze-dried reference sediment with mercuric chloride solution. Toxicity was detected at the mean value of 0.035 ppm level. The positive reference sediment was stable for a minimum of eighteen weeks.

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