Toxicity Assessment of Reference and Natural Freshwater Sediments with the LuminoTox Assay

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ABSTRACT: We examined the possibility of adapting the LuminoTox, a recently-commercialized bioanalytical testing procedure initially developed for aqueous samples, to assess the toxic potential of sediments. This portable fluorescent biosensor uses photosynthetic enzyme complexes (PECs) to rapidly measure photosynthetic efficiency. LuminoTox testing of 14 CRM (Certified Reference Material) sediments was first undertaken with (1) a "solid phase assay" (Lum-SPA) in which PECs are in intimate contact with sediment slurries for a 15 min exposure period and (2) an elutriate assay (Lum-ELU) in which PECs are exposed for 15 min to sediment water elutriates. CRM sediment toxicity data were then compared with those generated with the Microtox Solid Phase Assay (Mic-SPA). A significant correlation ($P < 0.05$) was shown to exist between Lum-SPA and Mic-SPA, indicating that both tests display a similar toxicity response pattern for CRM sediments having differing contaminant profiles. The sediment elutriate Lum-ELU assay displayed toxicity responses (i.e. measurable IC_{20}s) for eight of the 14 CRM sediments, suggesting that it is capable of determining the presence of sediment contaminants that are readily soluble in an aqueous elutriate. Lum-SPA and Mic-SPA bioassays were further conducted on 12 natural freshwater sediments and their toxicity responses were more weakly, yet significantly, correlated. Finally, Lum-SPA testing undertaken with increasing mixtures of kaolin clay confirmed that its toxicity responses, in a manner similar to those reported for the Mic-SPA assay, are also subject to the influence of grain size. While further studies will be required to more fully understand the relationship between Lum-SPA assay responses and the physicochemical makeup of sediments (e.g., grain size, combined presence of natural and anthropogenic contaminants), these preliminary results suggest that LuminoTox testing could be a useful screen to assess the toxic potential of solid media. © 2006 Wiley Periodicals, Inc. Environ Toxicol 21: 395–402, 2006.

Keywords: freshwater sediments; toxicity; LuminoTox; solid phase assay; photosynthetic enzyme complexes; sediment grain size

INTRODUCTION

Sediment contamination by (in)organic chemicals released in aquatic systems from diverse types of (non)point sources of pollution (urban, industrial, and agricultural) continues to be a crucial environmental issue. In the freshwater arena, biota can indeed be adversely affected by the resuspension of toxic sediments via both natural (e.g. flood scouring) and man-made (e.g., dredging, navigation, open water deposition) activities. Hence, management strategies invariably employ bioassays, along with chemical analysis, to appraise the toxic potential of contaminated sediments. This then

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leads to sound decision-making relating to sediment removal, disposal, or treatment based on considerations aimed at protecting aquatic species.

A fairly large number of bioassays can be used to measure the toxicity of sediment elutriates, pore waters, and extracts, but fewer are presently available to evaluate that of whole sediment where both readily-soluble and adsorbed toxicants may be present (Côté et al., 1998). Recommended whole sediment toxicity tests are usually performed with benthic organisms that measure growth and survival after 10 or 14 days of exposure (Borgmann et al., 2005; Péry et al., 2005). While these are undeniably useful and relevant bioassays, parallel research efforts have also focused on developing smaller-scale "direct contact" tests featuring shorter exposure times, as well as increased sample throughput and cost-effectiveness. Increasingly, such microscale assays are often used as prescreens to rapidly identify toxic sediment samples that can then be confirmed as such by the more traditional benthic organism tests. Of these so-called direct contact (or "solid phase") small-scale tests, all are exclusively conducted with bacteria (Kwan and Dutka, 1995; Bitton et al., 1996; Corbisier et al., 1996; Doe et al., 2005) or micro-algae (Blaise and Ménard, 1998; Adams and Stauber, 2004).

To augment solid media microscale testing possibilities, we examined the possibility of adapting the LuminoTox, a recently commercialized bioanalytical testing procedure initially developed for aqueous samples, to assess the toxic potential of sediments. The LuminoTox instrument is a portable fluorescent biosensor that uses photosynthetic enzyme complexes (PECs) to measure photosynthetic efficiency (Boucher et al., 2005; Environment Canada, 2005; LuminoTox, 2005). Intoxicated PECs yield markedly diminished photosynthetic efficiency over control PECs, thereby confirming the action of bioavailable contaminant(s). The LuminoTox analyzer thus offers a simple and rapid means of screening samples for the presence of toxicity. Herein, we report on the development of LuminoTox solid phase and elutriate assay procedures that were applied for testing (1) certified reference material (CRM) sediments and (2) natural freshwater sediments. The LuminoTox solid phase assay results were then compared with those generated with a well-standardized solid phase bacterial luminescence toxicity assay. The influence of sediment grain size on LuminoTox toxicity responses was also investigated.

MATERIALS AND METHODS

Sediment Samples

Two series of sediment samples were employed to generate toxicity data with the bioassay procedures described later. The first were certified reference materials (CRM) prepared by the National Water Research Institute (NWRI, Burlington, Ontario, Canada) comprising naturally-contaminated sediments originating from various locations in the Great Lakes basin (NWRI, 2000). These freeze-dried (ground to less than 200-mesh particle size) sediments have undergone chemical characterization for several PCB congeners, PAHs, chlorobenzenes, and a number of trace metals. We tested eight CRM sediments reported to be mostly contaminated with organics (EC-1 to EC-8) and six mainly contaminated with heavy metals (SUD-1, HR-1, TH-1, TH-2, WQB-1, and WQB-3). The second series of samples comprised 12 natural freshwater sediments reflecting mixed contamination and originated from the Saint-Lawrence River, Québec, Canada (M1 to M4), Lake Ontario, Ontario, Canada (B1 to B4), and the Berlin waterways (P1 to P4). These sediments were collected in spring-summer of 2003, homogenized/aliquoted in the laboratory, and quickly frozen ($-20°C$) for subsequent analyses linked to a different project. Subsamples were thawed and used to compare LuminoTox and Microtox solid phase assay toxicity results for the present study.

Finally, to determine whether the LuminoTox solid phase assay toxicity responses could be influenced by sediment particle size, toxicity testing was undertaken with a mixture of silica sand (Allwhite Silica Sand, medium fine grade No. 0; Shaw Resources, Nova Scotia, Canada, www.shawresources.ca; grain size between 0.125 and 0.25 mm) and kaolin clay (Fisher Scientific, catalogue No. K2-500, lot No. 9502120; grain size <0.004 mm). In this experiment, kaolin concentrations ranged from 0 to 100% sediment content.

Toxicity Testing Procedures

Bacterial Luminescence Solid Phase Assay

The Microtox solid phase assay (Mic-SPA) was performed to assess sediment toxicity by following the Environment Canada reference method (Environment Canada, 2002). The principle of the Mic-SPA is based on the fact that *Vibrio fischeri*, the luminescent marine bacterium employed in this assay, undergoes suppression of light output after short-term exposure to toxicants. Specific disposable polystyrene tubes (15.5×56 mm, 7.5 mL capacity, hemispherical bottom) and associated filter columns (Strategic Diagnostic Inc., catalogue No. AZF 686093) are required to undertake this assay. In brief, rehydrated bacteria placed in polystyrene tubes are directly exposed to a sediment sample (maximum dry/wet weight of 0.3 g and subsequent dilutions thereof in a saline diluent) for 20 min at 15°C. After exposure, bacteria are separated from the sediment fraction by pressing a filter column into the exposure tube where they are then concentrated in the filtrate and subsequently aliquoted to a reading tube. The light output of all tubes (sediment-exposed and controls) is measured with a photometer, after which inhibition percentages can be calculated.

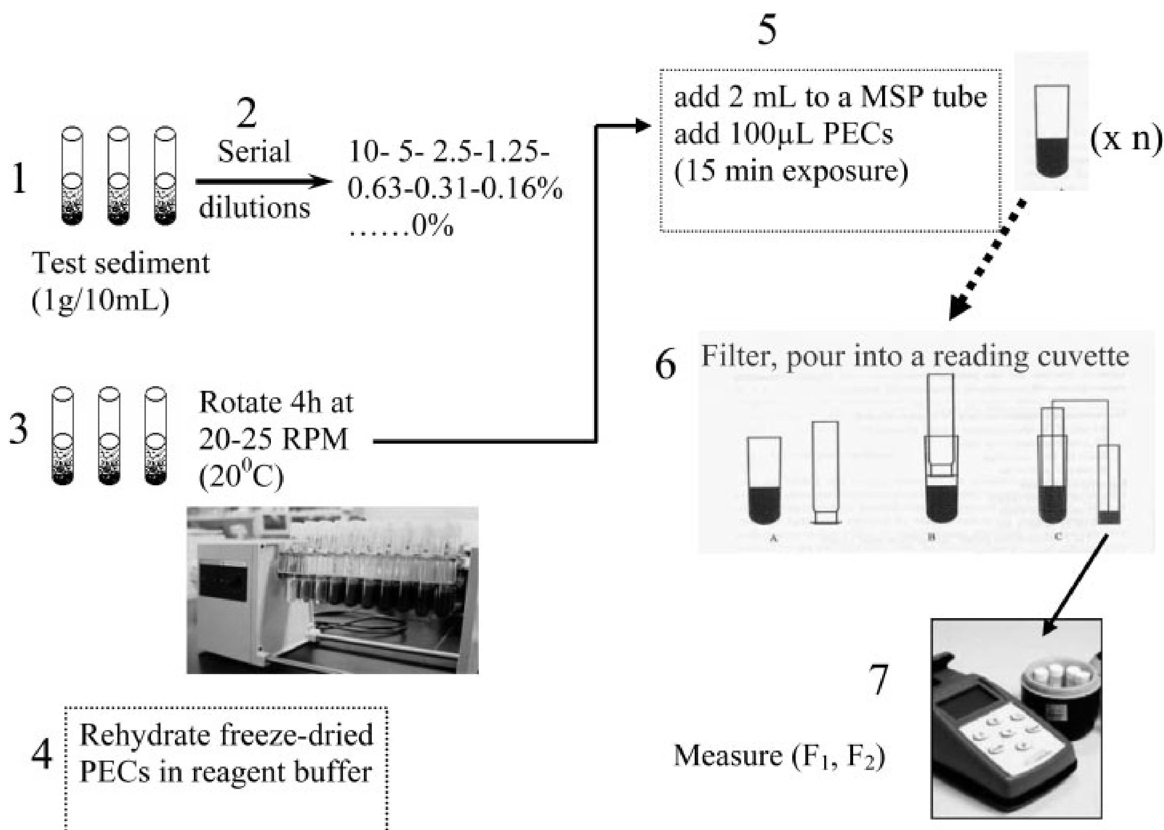

Fig. 1. Lum-SPA protocol developed with the LuminoTox Analyzer (refer to text in the Material and Methods section for details).

The determined endpoint is a 20 min IC_{50} (inhibitory concentration causing 50% light loss) expressed in % wt/v (g of wet or dry sediment per 100 mL of dilution water).

LuminoTox Assay Principle

The LuminoTox toxicity testing procedure makes use of stabilized photosynthetic enzyme complexes (PECs), isolated from spinach plant extracts. When PECs are challenged with chemicals or complex samples that can interfere with transmission of chlorophyll fluorescence linked to Photosystems I and II reaction sites, a corresponding decrease in fluorescence emission results (LuminoTox, 2005). The LuminoTox Analyzer, a special fluorometer programmed to measure photosynthetic activity based on production of chlorophyll fluorescence, can then measure the corresponding photosynthetic efficiency (Φ_p) of exposed and unexposed PECs. The analyzer measures Φ_p based on the following formula:

$$\Phi_p = (F_2 \text{ sample} - F_1 \text{ sample}) / F_2 \text{ blank}$$

F_2 fluorescence relates to fully reduced plastoquinone Q_a after rapid application of a high light intensity excitation of the PECs by the instrument, whereas F_1 relates to fully oxidized Q_a after corresponding application of a low light excitation to the PECs (LuminoTox, 2005). PEC fluorescence emissions for both F_1 and F_2 are measured at a wavelength >700 nm after light excitation at 470 nm. Percentages of inhibition based on exposure to different sample concentrations can then be calculated as follows:

$$\% \text{ inhibition} = [(\Phi_{\text{blank}} - \Phi_{\text{sample}})/\Phi_{\text{blank}}] \times 100$$

LuminoTox Solid Phase (Lum-SPA) and LuminoTox Elutriate (Lum-ELU) Assays

The experimental protocol developed for testing whole sediment toxicity with the LuminoTox Analyzer (Lum-SPA) is illustrated in Figure 1. A series of seven serial sediment dilutions (10, 5, 2.5, 1.25, 0.63, 0.31, and 0.16%) and controls (0%) were first prepared with Millipore Super Q water, in triplicate, in 20 × 150 mm borosilicate glass tubes (steps 1 and 2). All tubes were then sealed with Parafilm and placed in a vertical rotator (Caframo™ Reax 2 rotating mixer). Rotating speed was set at 20–25 rpm at room temperature (20°C ± 2°C) for 4 h (step 3). This mixing period is meant to maximize solubilization of hydrophilic contaminants (e.g. heavy metals), as well as of lower

molecular weight hydrophobic chemicals (e.g. naphthalene, acenaphthene), and it may, in turn, better expose more strongly hydrophobic chemicals (e.g. three-ringed or more PAHs, PCBs) associated with the sediment for subsequent contact with PECs. Freeze-dried PECs are then rehydrated in reagent buffer (containing 50 mM HEPES and 330 mM sucrose at pH 7.5) according to manufacturer's instructions (step 4) and allowed to stabilize for 15 min (LuminoTox, 2005). Immediately after vortexing individual glass tubes (starting with the 0% sediment-containing sediment tubes and ending with the 10% sediment tubes), a 2 mL-volume of control water (0% sediment) or of slurry (water-sediment mixture) is withdrawn from each tube and dispensed into a small plastic tube (identical to that used to perform the Mic-SPA). A 100 μL volume of PECs is then pipetted into each plastic tube for a 15 min exposure period (step 5). Afterwards, PECs are easily separated from the sediment fraction by pressing a filter column (identical to that used to perform the Mic-SPA) into the exposure tube. The PEC-containing filtrate contents ($\sim$2 mL) are next poured into a methyl acrylate (VWR Scientific Products, No. 58017-875) reading cuvette (step 6). Each cuvette is then placed into the LuminoTox Analyzer to obtain F_1 and F_2 readings, as well as photosynthetic efficiency (Φ_p) and percentages of inhibition, as described before (step 7). The determined endpoint is a 15 min IC_{50} (inhibitory concentration causing a 50% fluorescence inhibition related to photosynthetic efficiency) expressed in % of wet or dry sediment (g per 100 mL of dilution water).

The experimental protocol developed for testing sediment elutriate toxicity with the LuminoTox Analyzer (Lum-ELU) is performed with some variants as compared to Lum-SPA. A 25% (5 g/20 mL) sediment slurry is prepared, in triplicate, in 50 mL plastic tubes (Fisher Scientific, No. 14-961-33) with Millipore Super Q water. The three tubes were then capped and placed in a vertical rotator (Caframo™ Reax 2 rotating mixer). Rotating speed was set at 20–25 rpm at room temperature (20°C ± 2°C) for 4 h. This mixing period is meant to maximize solubilization of hydrophilic contaminants (e.g. heavy metals), as well as of lower molecular weight hydrophobic chemicals (e.g. naphthalene and acenaphthene), for later contact of the elutriate with PECs. Afterwards, the three 50 mL tubes are centrifuged (3000 rpm, 10 min) and a series of seven serial dilutions of each supernatant (25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39%) and controls (0%) are prepared in smaller tubes (any plastic or glass tube of 10–15 mL volume capacity is adequate for this purpose) with Millipore Super Q water as dilution medium. Contents of these tubes comprise the sediment elutriates from which 2 mL of each are then pipetted into another tube (e.g., Mic-SPA tubes) to which have just been added 100 μL of (rehydrated and stabilized) PECs.

TABLE I. Toxicity data (and selected chemical data) for certified reference material (CRM) sediments related to Lum-SPA and Mic-SPA assays

CRM Sediment	Lum-SPA IC_{50}[a] (% in g/100 mL)	Mic-SPA IC_{50}[a] (% in g/100 mL)	PAHs[b] (μg/g)	Cu[c] (μg/g)
CRM sediments mostly contaminated with organics (NWRI, 2000)				
EC-1	0.77 (0.66–0.91)	0.065 (0.057–0.073)	92.6	–
EC-2	1.14 (0.99–1.31)	0.22 (0.18–0.27)	20.1	–
EC-3	1.26 (1.07–1.50)	0.67 (0.59–0.75)	3.47	–
EC-4	0.58 (0.53–0.63)	0.052 (0.022–0.12)	6.74	–
EC-5	0.59 (0.48–0.73)	0.18 (0.12–0.27)	4.99	–
EC-6	1.65 (1.37–2.02)	0.75 (0.65–0.86)	1.97	–
EC-7[d]	3.20 (2.64–3.90)	>19.7[e]	1.23	–
EC-8	0.55 (0.48–0.61)	0.36 (0.32–0.41)	2.11	–
CRM sediments mostly contaminated with metals (NWRI, 2000)				
SUD-1	0.41 (0.34–0.51)	0.072 (0.02–0.28)	–	565
HR-1	0.80 (0.66–0.98)	0.088 (0.06–0.13)	–	78.5
TH-1	0.52 (0.44–0.61)	0.073 (0.05–0.11)	–	103.2
TH-2	0.67 (0.61–0.73)	0.053 (0.047–0.060)	–	123
WQB-1	0.94 (0.81–1.09)	0.080 (0.065–0.098)	–	78.3
WQB-3	1.06 (0.84–1.34)	0.25 (0.22–0.29)	–	82.9

[a] IC_{50} values with their 95% confidence intervals (in brackets).

[b] Total PAHs indicate the sum of the nine following compounds: Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[ghi]perylene, Benzo[a]pyrene, Benzo[a]anthracene, Fluoranthene, Indeno[123-cd]pyrene, Phenanthrene, Pyrene (NWRI, 2000)

[c] Based on total trace metal concentrations (NWRI, 2000).

[d] EC-7 datum was left out of the correlation analysis because of the indeterminate Mic-SPA value.

[e] Less than 50% effect noted at the maximum test concentration of 19.7%.

Following a 15 min exposure period, the content of each tube is poured into a reading (methyl acrylate) cuvette. Each cuvette is then placed into the LuminoTox Analyzer to obtain F_1 and F_2 readings, as well as photosynthetic efficiency (Φ_p) and percentages of inhibition, as described earlier. The determined endpoint is a 15 min IC_{20} (inhibitory concentration causing a 20% fluorescence inhibition related to photosynthetic efficiency) expressed in % of wet or dry sediment elutriate prepared from a 25% sediment slurry (i.e., 5 g of sediment per 20 mL of dilution water).

Data Analysis

Mic-SPA IC_{50}s and 95% confidence limits were calculated by regression analysis with an Omni™ software package (Version 1.18 or equivalent) marketed by Strategic Diagnostics (USA). Lum-SPA and Lum-ELU measurement endpoints (IC_{20}s, IC_{50}s, and 95% confidence limits) were calculated using a nonlinear regression procedure (REGTOX) that is available as an Excel macro at the following link: http://eric.vindimian.9online.fr. Correlation analysis between Mic-SPA and Lum-SPA or between any toxicity test and metal/organic contaminant data for the reference CRM sediments was determined using Pearson Product Moment or Spearman Rank Order tests with significance set at $P < 0.05$. Correlation analysis was performed with Statistica software (Version 5.5).

RESULTS AND DISCUSSION

CRM Sediments

Toxicity data generated with the Lum-SPA and Mic-SPA assays are shown in Table I. Of the 14 CRM sediment samples, only EC-7 failed to yield a reportable IC_{50} value with the Mic-SPA assay. This same CRM sediment yielded the highest Lum-SPA IC_{50} value and is also characterized by the lowest content in total PAHs. A significant correlation ($P < 0.05$) was shown to exist between Lum-SPA and Mic-SPA IC_{50}s (Fig. 2), indicating that both tests display a similar toxicity response pattern for a group of CRM sediments ($n = 13$) having differing contaminant profiles (NWRI, 2000). Additional analyses (correlation data not shown) also demonstrated marginally significant correlations between Lum-SPA with Cu ($r = -0.77$; $P = 0.072$), and also between Mic-SPA with Cu ($r = -0.71$; $P = 0.10$) for the group of CRM sediments mostly contaminated with metals (see Table I), as well as significant correlations between Mic-SPA and total PAHs ($r = -0.86$; $P = 0.002$) for the remaining eight CRM sediments mostly contaminated with organics (see Table I), suggesting that these two solid phase assays can respond differently to (in)organic classes of contaminants. No other significant correlation was found for this group of eight CRM sediments between either Lum-SPA and total PCBs or chlorobenzenes or between Mic-SPA and these same classes of chemicals.

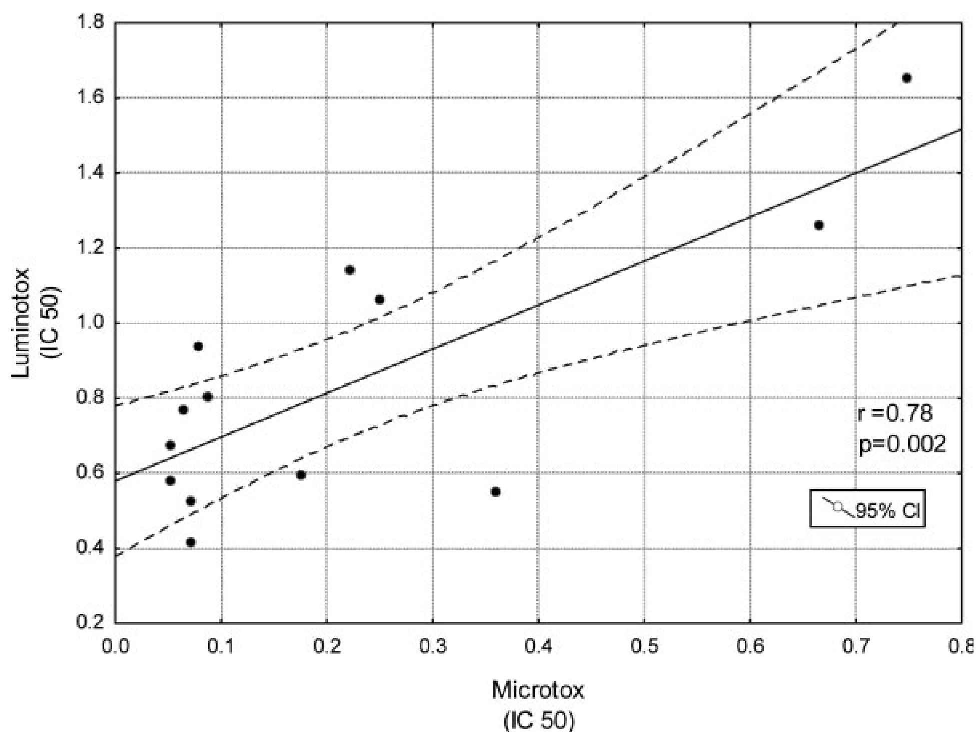

Fig. 2. Correlation analysis for Lum-SPA and Mic-SPA IC_{50}s after testing 13 CRM sediments. Endpoints are expressed in % wt/v (g of dry sediment per 100 mL of dilution water).

TABLE II. Toxicity data generated for certified reference material (CRM) sediment elutriates with the Lum-ELU assay

CRM-org Sediments[a]	Lum-ELU IC_{20}[b]	CRM-Met Sediments[c]	Lum-ELU IC_{20}[b]
EC-1	51.1 (35.7–68.0)[d]	SUD-1	0.75 (0.61–0.93)[d]
EC-2	91.6 (70.9–95.1)[d]	HR-1	>100
EC-3	>100	TH-1	49.4 (32.3–79.8)[d]
EC-4	>100	TH-2	80.6 (65.1–95.2)[d]
EC-5	>100	WQB-1	52.8 (37.3–70.1)[d]
EC-6	>100	WQB-3	51 (41.8–60.1)[d]
EC-7	>100		
EC-8	4.67 (2.05–9.8)[d]		

[a] CRM sediments mostly contaminated with organics (NWRI, 2000).
[b] 15 min IC_{20} (inhibitory concentration causing a 20% fluorescence inhibition related to photosynthetic efficiency) expressed in % of dry sediment elutriate prepared from a 25% sediment slurry (i.e., 5 g of sediment per 20 mL of dilution water).
[c] CRM sediments mostly contaminated with metals (NWRI, 2000).
[d] IC_{20} values with their 95% confidence intervals (in brackets).

Because LuminoTox testing on CRM sediment elutriates revealed few 50% inhibitory effects on PEC photosynthetic efficiency, we chose to report these responses via an IC_{20} to reduce the number of indeterminate toxicity values (i.e. $IC_{50} > 100\%$). With this more sensitive endpoint, the Lum-ELU assay displayed toxicity responses (i.e. measurable IC_{20}s) for eight of the 14 CRM sediments (Table II), suggesting that it is capable of determining the presence of sediment contaminants that are readily soluble in an aqueous elutriate. Three of 8 elutriates and five of 6 elutriates, prepared from CRM sediments mostly contaminated with organics (CRM-org sediments) and metals (CRM-met sediments), respectively, were shown to be toxic (Table II). Lesser "toxicity hits" obtained on the CRM-org sediment elutriates is not altogether surprising, since they are mainly contaminated with classes of hydrophobic chemicals (e.g. PAHs and PCBs) that do not easily become soluble in an aqueous medium. Indeed, no significant correlation was found between Lum-ELU IC_{20}s and total PAHs, total PCBs or chlorobenzenes for these eight CRM-org sediments (indeterminate IC_{20} values of ">100" in Table II were set at "100" for the purpose of this correlation analysis). In contrast and based on available CRM sediment metal data (NWRI, 2000), correlations were demonstrated between Lum-ELU IC_{20}s and As ($r = -0.77$; $P = 0.1$, marginally significant), between Lum-ELU and Cu ($r = -0.997$; $P = 0.001$), and between Lum-ELU and Ni ($r = -0.997$; $P = 0.001$) for the six CRM-met sediments. This again suggests that Lum-ELU testing is capable of detecting the toxicity of metals that are loosely adsorbed to these sediments. Of the six CRM-met sediments, HR-1 is the least contaminated in As, Cu, and Ni (NWRI, 2000), which may explain the nontoxic response of the Lum-ELU assay towards its elutriate.

Natural Freshwater Sediments

The Lum-SPA and Mic-SPA bioassays were further conducted on 12 sediments originating from lotic and lentic freshwater environments of Canada and Germany (see Materials and Methods section). As with the CRM sediments, their toxicity responses were again significantly correlated, suggesting that both tests answer to (at least some of) the same types of contaminants likely present in these sediments (Fig. 3). At this time, absence of information on the physicochemical makeup of these sediments (e.g., combined presence of natural and anthropogenic contaminants, grain size) precludes further interpretation. Sediment samples M3 and M4 (Fig. 3), likely influenced by municipal effluents because of the area in the Saint-Lawrence River where they were collected, exceptionally demonstrate that Lum-SPA and Mic-SPA toxicity responses are linked to different factors. Removing these from the correlation analysis would have resulted in an even better agreement between the two assays.

Influence of Sediment Grain Size on Lum-SPA Toxicity Responses

Test sediment grain size has been shown to be an important confounding factor interfering with toxicity responses in solid phase assays conducted with luminescent bacteria (Environment Canada, 2002). With the Mic-SPA assay, decreasing grain size causes a pronounced decrease in IC_{50}s (increased toxicity) when fines, defined as sediment

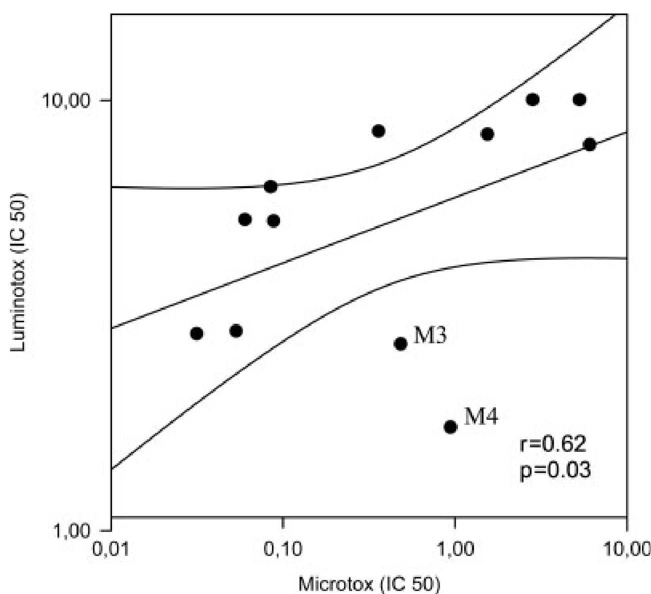

Fig. 3. Correlation analysis for Lum-SPA and Mic-SPA IC_{50}s after testing 12 natural freshwater sediments. Endpoints are expressed in % wt/v (g of wet sediment per 100 mL of dilution water).

TABLE III. Influence of sediment grain size on Lum-SPA assay toxicity responses

Experimental Mixture			
Kaolin (%)	Silica Sand (%)	IC_{50}	95% Confidence Intervals
0	100	20.5	11.0–53.7
5	95	14.0	11.0–23.1
10	90	13.7	10.3–20.9
20	80	4.7	3.8–6.4
40	60	4.0	2.6–5.9
60	40	3.1	2.3–3.9
80	20	3.0	2.6–3.5
90	10	2.2	1.9–2.4
95	5	3.7	3.3–4.1
100	0	2.4	1.9–4.3

IC_{50}s are expressed in % wt/v (g of dry sediment per 100 mL of dilution water).

particles ≤0.063 mm in size, increase within the sediment matrix (Ringwood et al., 1997; Tay et al., 1998). Indeed, Mic-SPA assays undertaken with 100% (uncontaminated) kaolin clay, where sediment particles are <0.004 mm in size, have been found to produce IC_{50}s ranging from 0.14 to 0.245% (Ringwood et al., 1997; Tay et al., 1998). These studies have prompted the establishment of guidelines to confirm the toxicity of sediment samples assessed by Mic-SPA assays, one of which is to consider toxic any sediment with an $IC_{50} < 0.1\%$, regardless of grain size (Environment Canada, 2002).

In this work, we conducted an analogous kaolin clay toxicity experiment with the Lum-SPA assay to ascertain whether its toxicity responses could also be subject to the influence of fines. In a manner similar to that of the Mic-SPA assay, Lum-SPA IC_{50}s displayed a sharp drop when the percentage of fines increased to 20% or more (Table III). At such concentrations of fines, IC_{50}s ranged from 2.17 to 4.72%. These results suggest that sediment samples appraised with the Lum-SPA protocol should only be considered toxic when their IC_{50}s are less than 2%, especially if sediment grain size has not been determined. Since CRM sediments were "ground to less than 200-mesh particle size" (NWRI, 2000), which corresponds to <0.074 mm, they can be considered as "fines" (as defined previously). Hence, based on a cut-off sediment toxicity criterion of <0.1% and <2% for Mic-SPA and Lum-SPA IC_{50}s, respectively, seven of 14 CRM sediments would be considered toxic owing to the presence of (in)organic contaminants according to the former assay, whereas 13 of 14 would be likewise considered according to the latter (Table I). Interpretation regarding the intrinsic toxicity of the 12 natural freshwater samples, based on Lum-SPA and Mic-SPA assays (Fig. 3), is presently not possible because grain size sediment data are not yet available. The generally higher IC_{50} values observed, ranging from 1.7% (sample M-4) to 8.3% (sample M-2) for the Lum-SPA assay (IC_{50} data not shown but linked to Fig. 3), suggest that most of these natural sediments have coarser grain sizes and that the above toxicity criterion of <2% for Lum-SPA would not apply.

Clearly, further studies will be required to more fully understand the relationship between LuminoTox solid phase responses and the physicochemical makeup of sediments (e.g., grain size, combined presence of natural and anthropogenic contaminants). Awareness of confounding factors will then allow application of specific guidelines for proper interpretation of test data that will adequately distinguish between natural and anthropogenic sediment toxicity. At this time, the preliminary results obtained with the Lum-SPA and Lum-ELU protocols intimate that LuminoTox technology appears to hold promise for screening the toxic potential of solid media.

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