

## Toxicity of Reference Sediment Samples

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**Abstract:** This study was done to determine toxicity of certified reference sediment to be used for quality assurance in sediment toxicity testing program. Certified Reference Harbor Sediment (HS-4B for PAHs) and Estuarine Sediment (LGC-6137 for Trace Metals) were procured commercially from LGC Company, UK, and repeatedly analyzed against solid phase Microtox toxicity assay to determine EC<sub>50</sub> values. Both PAHs rich and trace metals rich sediments were very toxic to Microtox with EC<sub>50</sub> 405 mg L<sup>-1</sup> and 554 mg L<sup>-1</sup> respectively. Solvent extraction resulted in a drastic decrease in the toxicity of PAHs rich reference sediment sample but in trace metal rich sediment the decrease in the toxicity was marginal. The certified reference sediments with the availability of toxicity data can be incorporated for quality assurance as controls in Microtox toxicity determination of field samples of unknown toxicity.

**Key words:** Reference sediment; microtox; toxicity; PAHs; trace metals

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### INTRODUCTION

Eco-toxicological testing of sediments began in the late 1970s, as a consequence of by-products from dredging operations<sup>[1]</sup>. Later, sediment contamination has become an integral part of sediment quality assessment. Developing valid methods for determining the extent and severity of such pollution is central to assessing environmental damage and guiding remediation efforts. This is often difficult due to distribution of contaminants among multiple phases varying in bioavailability and speciation effects, since different forms of chemical species in different compartments may vary in toxicity. Chemical analyses alone may not suffice to describe effects of chemicals present at contaminated site.

Environment Canada has been developing a rule based weight-of-evidence (WOE) approach for assessing the need for sediment remediation in areas of contamination. This approach uses four lines of evidence based on sediment chemistry, laboratory sediment toxicity, invertebrate community structure, and bioaccumulation. The underlying philosophy in the approach is that observations of elevated sediment contamination concentrations alone are not conclusive evidence of ecological degradation; it is the biological responses to these contaminants that are of primary concerns. These biological responses are represented by

both sediment toxicity and benthic invertebrate community structure where differences in faunal assemblages in contaminated areas compared to uncontaminated reference locations, indicate a deleterious impact resulting from sediment conditions<sup>[2]</sup>.

One of the basic trends in analysis and monitoring of environmental pollutants is the development of methods for the determination of analytes at increasingly lower concentrations in samples of very complex matrices. Economic and social implications of inaccurate data can be severe and therefore quality assurance (QA) is vital for environmental analysis. Microbial tests have been widely used in environmental toxicity screening due to similarity of complex biochemical functions in bacteria and higher organisms<sup>[3]</sup>. Microtox Solid Phase toxicity assay employing photobacterium *Vibrio fischeri* has been extensively used in sediment toxicity assessment<sup>[4-7]</sup>.

However, in sediment toxicity assessment research there is an inherent problem in obtaining reference sediment for comparison since no natural sediment is totally uncontaminated and has the same physical features as the chemically corrupted sediment and no two sediment samples are identical in nature. The best reference sediment is the natural sediment from the cleaner area of the study location. Therefore, it was felt necessary to get a sample that can be used as the

toxicity standard for quality assurance of data and also toxicity ranking. Kawn and Dutka<sup>[8]</sup> have used clean lake sediment as positive control and incinerator ash as negative control.

Reference material (RM), a material or substance whose one or more properties are sufficiently homogenous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to material. Certified reference material (CRM), a reference material accompanied by a certificate, whose one or more property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied an uncertainty at a stated level of confidence. Problems related to quality assurance for environmental monitoring has been comprehensively described. The international harmonized protocol for the proficiency testing of analytical laboratories resulted from co-operation of the International Standardization Organization. The system of quality assessment consist of, monitoring and evaluation of the precision of the results produced, based on periodical analysis of control samples; evaluation of accuracy by, analysis of certified reference materials, comparison of the results obtained by means of a given method and a reference method, analysis of samples after standard addition, carrying out interlaboratory studies, use of control charts, application of a special system of auditing.

In this study, certified standard reference sediment samples with known associated chemical contaminants have been procured from an international agency (LGC Company, UK). These certified samples are meant to be included in chemical estimation of PAHs and trace metals determination for quality assurance. We have used the same samples for toxicity assay by Microtox Solid Phase test and EC<sub>50</sub> with confidence range determined in order to see the feasibility of their inclusion in routine Microtox assay for quality assurance of field samples of unknown toxicity.

## MATERIALS AND METHODS

**Reference Sediment Samples:** Certified Reference Harbor Sediment (HS-4B for PAHs) and Estuarine Sediment (LGC-6137 for Trace Metals) were procured commercially from LGC Company, UK, and repeatedly

Table 1: PAHs in Reference Sediment Sample

PAH ( $\mu\text{g kg}^{-1}$ )	RHS-4B
Naphthalene	220.00
Methyl Naphthalenes	160.00
Biphenyl	40.00
Dimethyl Naphthalenes	-
Acenaphthylene	300.00
Acenaphthene	90.00
Trimethyl Naphthalenes	-
Fluorene	160.00
Methyl Fluorenes	-
Dibenzothiaphene	110.00
Methyl Dibenzothiaphenes	-
Phenanthrene	1910.00
Methyl Phenanthrenes	-
Dimethyl Phenanthrenes	-
Fluoranthene	3330.00
Anthracene	460.00
Pyrene	2550.00
Benzo(a)anthracene+Chrysene	3220.00
Benzo(b&k)fluoranthene	3320.00
Benzo(a)pyrene	1550.00
Indo(1,2,3-cd)pyrene	-
Dibenzo(a,h)anthracene	340.00
Benzo(g,h,i)perylene	1230.00
Dibenz(a,l)pyrene	160.00
Coronene	310.00
Total PAHs	19460

analyzed against solid phase Microtox toxicity assay to determine EC<sub>50</sub> values.

**PAHs Contaminated Reference Sediment:** The HS-4B samples were commercially procured from LGC Company, UK. The samples were collected from Nova Scotia. Environments at these sites reflect varying degrees of commercial and industrial activity. The material has been freeze dried, passed through a No. 120 (125  $\mu\text{m}$ ) sieve, homogenized in a modified cement mixer, then sub sampled into 100 gram lots and placed in solvent-rinsed, pint-sized steel cans. The samples consists of a suite of PAHs whose values of concentrations range from approximately 0.2  $\text{mg kg}^{-1}$  to 60  $\text{mg kg}^{-1}$  of dry sediment. Homogeneity tests and certification experiments of PAHs on these materials were carried out using 35 gram sub sample. Determination were carried out using capillary column gas chromatography with flame ionization detection (GC-FID), capillary column gas chromatography with mass spectrometric detection, high performance liquid chromatography with ultraviolet absorption detection (HPLC-UV), high performance liquid chromatography with fluorescence detection (HOLC-FLD), high performance liquid chromatography with mass spectrometric detection (HPLC-MS). PAHs levels in HS-4B are given in Table 1. The total PAHs in HS-4B were 19460  $\mu\text{g kg}^{-1}$ . Considering different types of

Table 2: Trace Metals in Reference Sediment (LGC-6137)

Trace Metals	Levels, mg kg <sup>-1</sup>
As	12.40
Cd	-
Cr	47.00
Cu	31.60
Fe	30700
Mn	665.0
Ni	31.50
Pb	73.00
V	47.00
Zn	231.0

PAHs it was found that high molecular weight PAHs were extremely high in Reference Harbour Sediment as compared to low molecular weight compounds (Table 1). Among the high molecular weight compounds almost all the compounds, fluoranthene, benzo(a) anthracene+ chrysene, benzo(b&k)fluoranthene, benz(a) pyrene and pyrene were high. Among low molecular weight PAHs naphthalenes and phenanthrenes were more abundant than fluorene and dibenzthiaphene.

#### Trace Metals Contaminated Reference Sediment:

This material is an estuarine sediment that was obtained from Severn Estuary offshore the heavily industrialized area around Avonmouth. The material was dried and sterilized at 130°C for three hours and ground to less than 200 µm particle size. The material was then homogenized, divided in to 50g sub-samples and packaged in amber glass bottles with screw caps. The material was tested for homogeneity by analyzing randomly selected samples for the analytes of interest. The material was judged to be homogeneous as the variation between the samples tested was not significantly greater than the analytical variation. The material has been tested for the stability by analyzing randomly selected samples which have been stored at various temperatures (20 and 40°C) over various periods of time. No evidence of instability was observed. This material was certified by means of an inter-laboratory exercise. This involved the analysis of samples of the material by the participants using methods of their own choice. The certified values are based on the mean of laboratory means following elimination outlying results. The uncertainties were calculated by combining the 95% confidence intervals obtained from the inter-laboratory exercise with the uncertainties calculated from homogeneity study. The extractable metals content refers to metals soluble in hot Aqua Regia using methods based on ISO 11466 (1995). Analytical techniques employed by the participants included ICP-OES, ICP-AES, AASS and

GFAA, with hydride generation for some elements. The levels of various trace metals in LGC-6137 are given in (Table 2). The sample contained 30700 ppm iron which was highest among other metals, followed by manganese 665 ppm, zinc 231 ppm, lead 73 ppm, vanadium and chromium 47 ppm, copper 31.6 ppm, nickel 31.5 ppm and arsenic 12.4 ppm.

#### CLEANING OF REFERENCE SEDIMENT

A portion of the PAHs rich reference sediment sample(HS-4B) and trace metals rich reference sediment sample (LGC-6137) was extracted with the solvents of varying polarity in Soxhlet extraction to remove organic contaminants. The extraction was done over night with hexane followed by over night extraction with dichloromethane and over night extraction with methanol.

#### Bacterial Assay (Microtox Solid Phase Toxicity Assay):

The Microtox Toxicity Test System records the light output of luminescent bacteria, *V. fischeri* (Microtox reagent) before and after exposure to test samples and process the raw data statistically to produce reports on the toxicity of the samples. Microtox Reagent contains freeze dried bacteria *Vibrio fischeri* 4%, with salt sodium chloride 2%; and nutrients Skim milk solid 94% and reconstituted immediately before use. Microtox Analyzer Model 500 is integrated with a Microtox Data Collection and Reduction System (Data System). The data collection and reduction system comprises an IBM or fully IBM compatible computer that interface directly with the Microtox Analyzer via a standard RS-232 serial port. The microtox software contains all of the program for collecting storing and processing microtox test data. The software is factory loaded on microtox data system (Microtox® updated Manual)<sup>[9]</sup>.

In Solid Phase Microtox Test (SPT) assay the bacteria is placed directly in close vicinity of solid particles and its response reflect in totality the action of toxicants along with synergists and antagonists present in a given sample (Kawn and Dutka)<sup>[8]</sup>. The assay protocols were as given in Microtox Manual<sup>[9]</sup>. A 300 mg of sediment sample weighed out into SPT tube. In the case of assay in fresh samples if the sample was wet liquid phase removed after centrifugation and solid phase mixed thoroughly to restore homogeneity. Large particles were avoided. In freeze dried samples <500 µm fraction was assayed by taking 300 mg of sample. To the sediment samples 3 mL microtox diluent was added and the tube covered with paraffin film and mixed by

Table 3: Solid Phase Microtox Toxicity Assay of PAHs rich Reference Sediment (HS-4B)

Sample Replicates	Solid Phase Microtox Toxicity Assay		
	EC <sub>50</sub> (mg/L)	95% CL	Toxicity Rating
1	514	344-768	VT
2	602	447-812	VT
3	434	329-572	VT
4	523	339-807	VT
5	262	173-394	VT
6	432	269-695	VT
7	349	231-527	VT
8	330	184-591	VT
9	327	178-598	VT
10	319	171-594	VT
11	302	188-485	VT
21	470	327-675	VT
Average	405	265-626	

NT=not toxic, MT=moderate toxic, T= toxic, VT=very toxic

thorough shaking using Vortex Genie mixer to completely dissociate and suspend the sample. Before serial dilution the sediment suspension was mixed by filing and dispensing 5-6 times, then while solids were suspended 15-tube serial dilutions were made in microtox diluent. Using repeat pipette 20 µL Microtox reconstituted reagent was added into each SPT tube and mixed by filling and dispensing for 5-6 times. After 20 min incubation at 15 °C filter column were inserted and pushed gently downward and 500 µl of filtrate was transferred to cuvette and read in Microtox Analyzer.

The data were captured in computer and EC<sub>50</sub> along with 95% confidence limit determined by the software provided with Analyzer.

The toxicity rating of sediment was done according to Kawn and Dutka<sup>[8]</sup> and the samples were categorized very toxic if the Microtox EC<sub>50</sub> was ≤ 5000 mg L<sup>-1</sup>; moderately toxic at EC<sub>50</sub> >5000-≤10000 mg L<sup>-1</sup>; and non toxic at EC<sub>50</sub> >10000 mg L<sup>-1</sup>.

## RESULTS AND DISCUSSION

Toxicity of PAHs Rich Reference Sediment. The toxicity of PAHs rich reference sediment sample (HS-4B) was determined by the solid phase Microtox assay wherein the bacteria is placed directly in close vicinity of solid particles and its response reflect in totality the action of toxicants along with synergists and antagonists present in a given sample. Microtox solid phase toxicity in the reference sediment sample was determined in twelve replicates and assay revealed an average EC<sub>50</sub> 405±0.85σ mg L<sup>-1</sup>, where standard

Table 4: Solid Phase Microtox Toxicity Assay of Trace Metals Rich Reference Sediment (LGC-6137)

Sample Replicates	Solid Phase Microtox Toxicity Assay		
	EC <sub>50</sub> (mg/L)	95% CL	Toxicity Rating
1	197	129-300	VT
2	325	181-582	VT
3	346	154-772	VT
4	603	239-1519	VT
5	654	443-964	VT
6	455	276-748	VT
7	302	22-421	VT
8	984	552-1753	VT
9	694	405-1192	VT
10	980	618-1553	VT
11	681	463-1002	VT
12	432	329-567	VT
Average	554	317-947	

NT=not toxic, MT= moderate toxic, T= toxic, VT=very toxic

deviation σ was 106, (ranging from 262-602 mg L<sup>-1</sup>) and the data are given in Table 3.

Toxicity of Trace Metal Rich Reference Sediment. The reference sediment rich in trace metals (LGC-6137) was determined by solid phase Microtox assay and twelve replicates of the sample were analyzed. The average EC<sub>50</sub> obtained was 554±0.82σ mg L<sup>-1</sup>, where standard deviation σ was 257, (ranging from 197-980 mg L<sup>-1</sup>), which showed that the sample was extremely toxic to Microtox (Table 4).

**Effect of Solvent Extraction on the Toxicity of Two Reference Sediments:** The PAHs rich sediment (HS-4B), and trace metal rich reference sediment (LGC-6137) were subject to exhaustive extraction in soxhlet with solvents of increasing polarity (hexane, dichloromethane and methanol) to remove organic contaminants from the sediment. The solvent extracted sediments were again subjected to toxicity determination and the data are reported in Table 5 and Table 6.

Toxicity of PAHs Rich Reference Sediment-Solvent Extracted. The toxicity of solvent extracted high PAHs contaminated sediment was determined in twelve replicates of the sample and the EC<sub>50</sub> was found to be dramatically increased to 23158±0.73σ mg L<sup>-1</sup>, where standard deviation σ was 1641, ranging from 19179-25130 mg L<sup>-1</sup> (Table 5).

Toxicity of Trace Metal Rich Reference Sediment-Solvent Extracted. The solvent extracted reference sediment rich in trace metals was tested for toxicity in twelve replicates and the EC<sub>50</sub> was found to be 1148±0.73σ mg L<sup>-1</sup>, where σ was 479, ranging from 699-2359 mg L<sup>-1</sup> (Table 6). All the sample replicates indicated that the toxicity varied around its mean ± σ.

Table 5: Solid Phase Microtox Toxicity Assay of PAHs rich Reference Sediment-Solvent Extracted (HS-4B-SE)

Sample Replicates	Solid Phase Microtox Toxicity Assay		
	EC <sub>50</sub> (mg/L)	95% CL	Toxicity Rating
1	24800	17070-36030	NT
2	19179	16490-22290	NT
3	21600	19640-23750	NT
4	23130	20890-25260	NT
5	23490	15990-34500	NT
6	22360	13880-36000	NT
7	24570	16440-36710	NT
8	23430	15500-35420	NT
9	23650	14810-37760	NT
10	25130	16800-37580	NT
11	24200	15310-38250	NT
12	22360	14502-37653	NT
Average	23158	16443-33433	

NT=not toxic, MT=moderate toxic, T= toxic, VT=very toxic

Table 6: Solid Phase Microtox Toxicity Assay of Trace Metals Rich Reference Sediment-Solvent Extracted (LGC-6137-SE)

Sample Replicates	Solid Phase Microtox Toxicity Assay		
	EC <sub>50</sub> (mg/L)	95% CL	Toxicity Rating
1	837	412-1699	VT
2	765	209-2801	VT
3	938	509-1727	VT
4	1211	579-2530	VT
5	1073	487-2362	VT
6	1055	685-1624	VT
7	585	276-1238	VT
8	699	291-1680	VT
9	1335	554-3216	VT
10	1520	846-2728	VT
11	1405	694-2844	VT
12	2359	1027-5420	VT
Average	1148	547-2484	

NT=not toxic, MT= moderate toxic, T= toxic, VT=very toxic

During recent years, the awareness of quality assurance and quality control in environmental analyses has constantly increased especially due to the implementation of new guidelines and regulations at both the national and international level. Strict quality control (QC) measures must be taken to ensure that high quality results are generated in the laboratory. An important role in the quality control is played by reference materials (RM), i.e., a material or substance whose one or more properties are sufficiently homogenous and well established to be used for the assessment of a measurement method. Achieving comparable results by using certified reference materials is one of the primary concerns of the scientific community. As a result, there is a growing demand for certified reference materials to cover different matrices and pollutants. Moreover, these

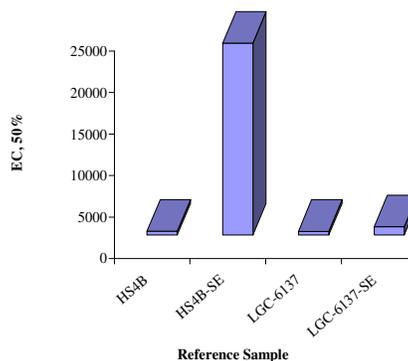


Fig. 1: EC<sub>50</sub> of Reference Sediment Samples HS-4B and LGC-6137 (before and after solvent extraction)

CRMs should be in close relationship to the determinants and target concentrations required by environmental bodies<sup>[10]</sup>.

Like in chemical analysis a need for reference sediment has been strongly felt, to be included in toxicity determination for quality assurance. Many studies have been done to make reference sediment sample or formulated sediment sample to be used as controls in toxicity assay<sup>[11-12]</sup>. Such sediments are also required for toxicity testing in which a chemical is spiked into sediment. Suedel and Rogers<sup>[13]</sup> identified potential advantages of formulated sediment may have over field collected reference sediment. There are some concerns that the variability and nature of field sediments can cause effects in addition to the toxicity of test material.

Also, concerns about use of field collected natural sediment include the difficulty in finding uncontaminated sediments readily available at nearby sites. Frequently reference sediments possessing sediment characteristics similar to those of the sediment are not readily available at nearby locality for use in sediment toxicity test. The best alternative may be provided if the contaminated sediment can be cleaned because it may retain most of the component and the texture.

This approach was used by Kawn and Dutka<sup>[14]</sup>, who prepared a non-toxic sediment by extensive washing and sieving. However, we proposed on the basis of present study that reference sediments prepared for chemical estimation can be conveniently included in Microtox assay for quality assurance. The advantage is their commercial availability from same source and interlaboratory comparison will be easily possible.

The two samples showed comparable toxicity and categorized as very toxic samples. It was interesting that on solvent extraction the EC<sub>50</sub> was increased by 2-fold in trace metals rich reference sediment (LGC-6137) but a dramatic increase of 57-fold was found in PAHs rich reference sediment sample (HS-4B). The total loss in toxicity in PAHs rich reference sediment sample on solvent extraction confirms that PAHs were the causative factor in this reference sediment. Where as marginal decrease in toxicity after organic solvent extraction of trace metal rich sediment sample shows that organic contaminants were less responsible for over all toxicity of this sample (Fig. 1). It is also indicated that Microtox assay is equally suitable to organic and inorganic contaminants.

### CONCLUSION

In the present study a certified reference sediment (HS-4B) sample with high contamination of PAHs and another certified reference sediment (LGC-6137) rich in trace metals contamination were subjected to extraction with organic solvents of increasing polarity. These reference sediments were chemically well characterized and are meant to be incorporated in the chemical analysis of sediment as controls for PAHs and trace metals for quality assurance determination. With the availability of toxicity data these sediments can be incorporated as controls in Microtox toxicity determination of field samples of unknown toxicity.

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