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Battery of Tests Approach Applied to Three Different Types of Sediment Extracts

B.J. Dutka A. Jurkovic R. McInnis
K.K. Kwan
Rivers Research Branch
National Water Research Institute
Environment Canada, P.O. Box 5050
Burlington, ON L7R 4A6 Canada

T. Murphy
Lakes Research Branch
National Water Research Institute
Environment Canada, P.O. Box 5050
Burlington, ON L7R 4A6 Canada

Abstract

Over the past eight years we have been evaluating a variety of sediment extraction procedures and have finally settled for a three phase sequential procedure which involves pore water extraction followed by Milli-Q water extraction. Then the dewatered sediment is extracted by a solution containing 10% methanol, 10% DMSO and 80% Milli-Q water. This three phase sequential extraction procedure was applied to Hamilton Harbour sediments and the extracts were tested for toxicant activity by the battery of tests approach. Based on these samples, it would appear that pore water bioassay results are probably most indicative of the bioavailable toxicants load in Hamilton Harbour sediments.

Introduction

In previous publications, Dutka et al. [1, 2, 3] described the results of studies to evaluate the suitability of various microbiological, biochemical and bioassay tests to become part of a "battery of test procedures" which could be used to designate, nationally and internationally, water bodies or sediments that are degraded or are being degraded due to toxic chemical discharges, or excessive nutrient inputs. This "battery of tests" could also be used to monitor the effectiveness of remedial actions or the effect of specific discharges on ambient riverine or lacustrine ecology.

For the majority of bioassay procedures the toxicants or genotoxicants must be in a liquid which, at the concentration used, is nontoxic itself and does not respond synergistically with the contained toxicants or genotoxicants. Chemists have many procedures for extracting specific toxicants or genotoxicants from solid phase samples, most of which are very time consuming and specific. Researchers and users of bioassay procedures, on the other hand, are interested in obtaining a general overall picture of the solid phase toxicant or genotoxicant loads after which more specific chemical extractions for bioassay testing can be carried out, if necessary.

Over the past eight years, we have been evaluating a variety of sediment extraction procedures. We have finally settled on a three phase sequential procedure, which does not extract each contaminant individually, however it does appear to provide an almost complete picture of the water soluble contaminants and a reasonable slice of the bioavailable organic contaminants.

In this paper we present the results of applying the three phase sequential extracting procedure to Hamilton Harbour sediments which contain a great variety of contaminants [4, 5] and then applying the battery of tests approach to these extracts.

Methods and Materials

Samples and Sample Collection

Sediments 3.1, 3.2, 3.3 (Figure 1) were collected as cores ranging in depth from 60–100 cm. Upon collection each core was homogenized and sealed in a polypropylene bucket and stored from September 26 to October 4, 1990 at 2 °C, (melting ice). After that they were kept at 4 °C (refrigerator) until extracting procedures were initiated.

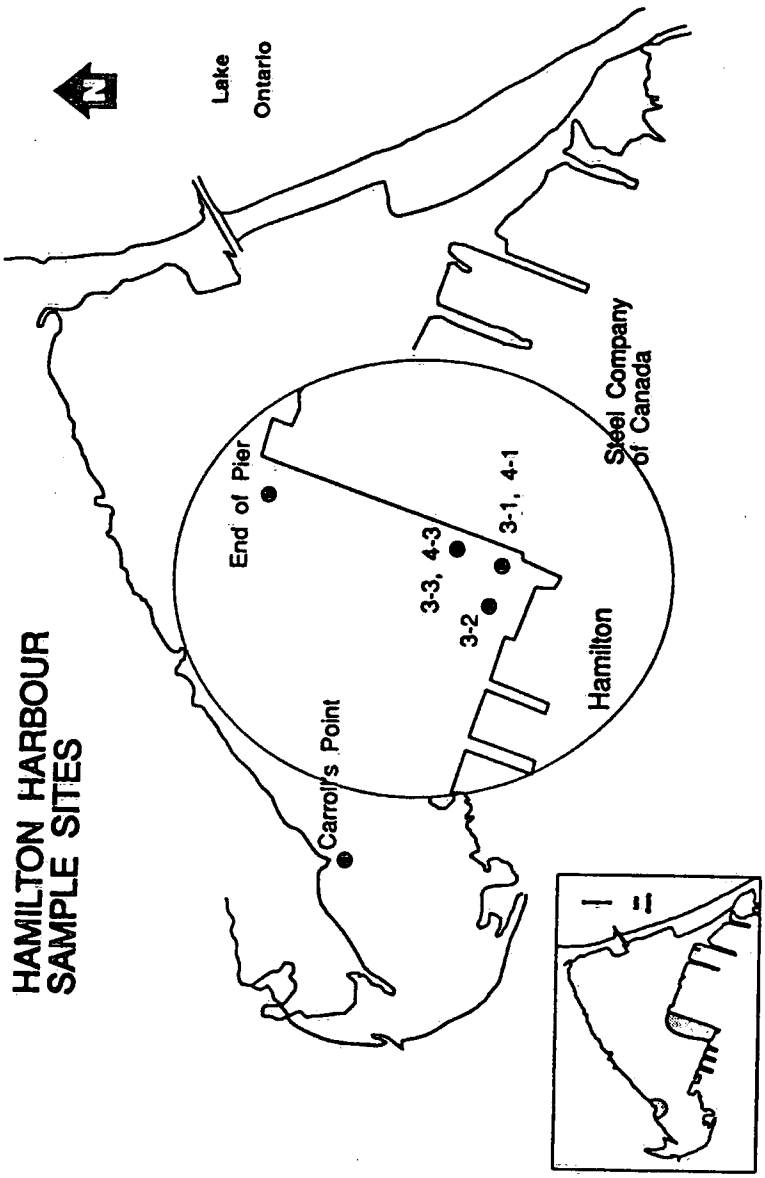

Figure 1: Locations of Hamilton Harbour sample sites.

Table 1: Site, description and Shepard classification for sediments from Hamilton Harbour.

Site	Sand	Silt	Clay	Classification
3.1	3.71%	65.52%	30.77%	Clayey silt
4.1	8.31%	61.74%	29.95%	Clayey silt
3.3	33.46%	33.85%	32.69%	Sand-silt-clay
4.3	18.58%	44.15%	37.29%	Clayey silt
3.2	6.22%	71.91%	21.86%	Clayey silt

Sediments 4.1, 4.3, Hamilton Harbour clean sediment (off Carrolls Point), and Hamilton Harbour end of Stelco pier, were collected with an Ekman dredge and each sample was thoroughly homogenized, then placed in a polypropylene bucket and stored from date of collection October 29, 1990, to November 1, 1990 at 2 °C, (melting ice). After that they were kept at 4 °C until extracting procedures were initiated. Samples 3.3 and 4.3 and 3.1 and 4.1 were collected near the same site.

On October 31, a sediment sample was collected from Honey Harbour (Georgian Bay) using an Ekman dredge. The sample was homogenized and placed in a polypropylene pail and maintained at 4 °C until extraction was completed on December 18, 1990.

Sediment size distribution and analytical procedures involved in this process are described by Duncan [6] and the results are shown in Table 1.

Sediments were extracted by three sequential procedures for bioassay testing by the battery of tests approach. The first procedure was to collect the pore (interstitial) water. The sediment was centrifuged for twenty minutes at 10,000 rpm at 4 °C and the supernatant was collected as the pore water. The dewatered sediment was weighed and an equal weight to volume of Milli-Q water was added, mixed and thoroughly shaken for three minutes and then centrifuged again for twenty minutes at 10,000 rpm. This supernatant was the Milli-Q extract and was used in the various bioassays. The dewatered sediment was weighed, and a volume of 10% DMSO (dimethyl sulfoxide) plus 10% methanol equivalent to the gram weight of the dewatered sediment was added to the sediment and the mixture was again shaken vigorously for three minutes. After shaking, the mixture was centrifuged at 10,000 rpm in

a refrigerated centrifuge for twenty minutes. After centrifugation the supernatant was removed and placed into acid-washed, Milli-Q water-rinsed brown screw capped bottles and frozen at -60 °C until tested. The solvent extract was tested at the 1% or less level (diluted with Milli-Q water). Prior to conducting any bioassays, the maximum allowable concentration (MAC) of the organic solvents has to be established. The MAC is defined as the concentration (%) of solvent that does not produce an effect on the test organisms [7].

Bioassays

Pore water, Milli-Q water extracts and 10% DMSO plus 10% methanol extracts were tested by the battery of tests approach [8]. The following bioassays were used to test the above extracts: Microtox, ATP-TOX System, SOS Chromotest, *Spirillum volutans*, *Daphnia magna*, *Ceriodaphnia dubia*, seed germination and root elongation (Buttercrunch lettuce), and nematode (*Panagrellus redivivus*) [3, 9].

Results and Discussion

Table 1 provides a description of the five contaminated sediments (3.1, 3.2, 3.3, 4.1 and 4.3) which were to be compared. From the sediment descriptions it can be seen that samples 3.3 and 4.3 which were collected from the same area have different sand and silt compositions from the other sediments, as well as showing some variability between themselves in their sand and silt composition. However, the variability in sediment structure seen in these Hamilton Harbour samples is much less than we have observed in river sediments [10] and tends to confirm our earlier observation that lake sediments tend to be more homogeneous than river sediments [11].

Table 2 shows the results of the bioassay tests on the pore waters collected from the eight sediments. The Stelco end of pier sediment is one of the least toxic sediments based on the bioassays used. Of all eleven tests applied to this sample, only the nematode test showed a slight response. Hamilton Harbour (H.H.) pore water 3.3 was found to contain the greatest toxicant load of all eight pore water samples.

Specifically reviewing each set of test results it can be seen that the Microtox test was positive in only two pore water samples H.H. 3.3 and 4.1, with H.H. 3.3 indicating the greater toxicant load (effect). Only three samples, the three controls, Honey Harbour, Stelco end of pier and H.H. Carrolls Point

Table 2: Results of bioassay tests on sediment pore water.

Site[a]	Micro-tox[b]	SOS[b] +S9	SOS[b] -S9	*C. dubia*[b]	Nematode[b] % A	Nematode[b] % B	*D. magna*[b]	*Spirillum volutans*[b]	Seed[b] %	Root Length[b]	ATP-TOX[b]	Tot. Pts.
Hny. H.	N.D.[c]	0.75	1.09	N.D.	91	81.9	N.D.	N.D.	100	N.D.	23.8	3
St. EOP	N.D.	0.89	1.09	N.D.	88	84.7	N.D.	N.D.	100	N.D.	8.3	3
H.H.CP	N.D.	1.12	0.73	N.D.	73	92.6	N.D.	N.D.	89.4	N.D.	32.3	6
H.H. 3.1	N.D.		1.09	0.1%	86	80.3	60%	+	100	54.2	43.5	31
H.H. 4.1	47.8		1.12	N.D.[d]	72	88.5	12%	+	100	55.3	44.3	22
H.H. 3.3	6.89		1.08	N.D.[b]	0	0	0.035%	+	94.1	31.2	59.3	46
H.H. 4.3	N.D.		1.09	N.D.[b]	88	0	25%	+	100	N.D.	48.3	28
H.H. 3.2	N.D.		0.98	N.D.[b]	88	92.9	50%	+	100	53.9	28.9	13

[a] Abbreviations: Hny H. is Honey Harbour; St. EOP is Stelco end of pier; H.H. is Hamilton Harbour.

[b] Microtox: EC_{50}% mL; SOS induction factor +S9, -S9; *C. dubia*: percent of the sample showing reproduction inhibition; Nematode: A: percent surviving; B: percent maturing; *D. magna*: EC_{50} as a percent of the sample; *Spirillum volutans*: 120 minute test; Seed test: percent germination; Root length percent inhibition; ATP-TOX system percent inhibition.

[c] N.D. is not detected.

[d] Samples very toxic, after toxicity diluted out no chronic toxicity noted.

were tested by the SOS Chromotest with S-9 addition, which tests for promutagens or chemicals requiring enzyme activation to express their effect, and these samples were negative. All the pore water samples were tested by the SOS Chromotest without S-9, for direct acting genotoxicants, and all the samples produced a negative response, indicating that either there were no genotoxicants present or the concentrations were too low to cause a response in the test.

The *Ceriodaphnia dubia* test which screens for the presence of chemicals producing chronic toxicity, was positive in only one sample, H.H. 3.1. However H.H. 4.1, 3.3, 4.3 and 3.2 pore waters were very toxic and after diluting the samples to nullify the acute toxicity effect, the chemicals able to induce a chronic toxicity effect, if present were also diluted out.

In the nematode test, each of the control samples produced a small but observable effect, either in reducing the number of survivors or in inhibiting the maturation of the survivors, a genetic process. The H.H. 3.3. sample produced the greatest effect in that no nematodes survived the test and thus no mature nematodes were produced. The results observed in H.H. 4.3 pore water were almost as striking as those seen in H.H. 3.1, here 88% of the nematodes survived but none of the survivors were able to reach maturity, indicating the presence of a genetic effect. The other three test sites H.H. 3.2, 4.1 and 3.1 produced results similar to those from the control sites.

The *Daphnia magna* results were very clear cut, the three control sites were negative and the five tested sites were all positive for toxicants. Pore water H.H. 3.3. produced the most toxic effect observed in this laboratory; that is, 0.035% of the original pore water sample was capable of producing an EC_{50} effect, (50% of the animals dying within 48 h).

The *Spirillum volutans*, test which is based on a 120 minute contact period, produced results very similar to the *D. magna* test. Pore waters H.H. 3.3 produced a positive (toxic) result in 10 minutes and H.H. 4.3 was positive in 60 minutes while the other three test sites were positive within 120 minutes.

In the seed germination and root elongation test there were no strong toxic effects inhibiting seed germination; however, in the root length portion of the test, four of the five test site pore waters produced root length inhibition with the greatest effect being seen in H.H. 3.3.

The ATP-TOX System, which is usually the most sensitive toxicity screening test, showed the greatest toxic response in pore water H.H. 3.3 followed closely by pore waters H.H. 4.3 and then H.H. 3.1 and 4.1.

A point-ranking scheme, based on the degree of toxicant or genotoxicant activity shown in each bioassay, was used to rank each sample in relation to its bioavailable toxicant load [8]. In this scheme the greater the point total, the higher the ranking and, thus, the more toxic the sample. Application of this point-ranking scheme to Table 2 bioassay responses indicates that H.H. 3.3. sediment pore water contained the greatest concentration of toxicants and genotoxicants, with H.H. 3.1 and H.H. 4.3 being the next most toxic sites. The control sites show very similar extremely low point ratings, and their data contrast well with the expected toxic samples. As noted earlier, the Stelco end of pier pore water was one of the least toxic control sediments tested.

Results of the Milli-Q water extraction tests are presented in Table 3. The Milli-Q extraction process, is believed to extract some of the more firmly bound chemicals from the sediment particles (organic and inorganic) and together with the pore water, should provide an indication of the bioavailability of all the water soluble toxicants in these sediments. Comparing Table 2 and 3 point scores, it can be seen that most of the pore water samples (five out of eight) were slightly more toxic than were the Milli-Q water extracts, two samples produced the same point scores and one sample indicated a slightly greater toxicant load in the Milli-Q water (Honey Harbour). Like that seen with the pore water extracts, H.H. 3.3 sediment was the most toxic and the Stelco end of pier data again indicated that a water extract of this sediment was not very toxic to the bioassays used.

Highlights of Table 3 are the nematode and *Daphnia magna* results. All the Milli-Q water samples indicated a response to one or both parts of the nematode test with Milli-Q extracts of H.H. 3.3 and H.H. 4.3 showing the greatest inhibition effects. In H.H. 3.3, 34% of the nematodes survived the 96-h test, but of those surviving only 0.8% reached maturity, while in H.H. 4.3, 96% of the nematodes survived but none reached maturity. These results confirm Table 2 observations.

The *Daphnia magna* acute toxicity test with Milli-Q water extracts produced very similar results to those seen with pore water in Table 2. Hamilton Harbour 3.3, 4.3, and 4.1 had the greatest toxicant effects. The Stelco end of pier Milli-Q water extract produced a very minor response in the *D. magna* test; that is, the unconcentrated, undiluted sample was able to kill 20% of the test animals in 48 hours.

Only four bioassays were performed on the solvent extracts from the eight sediments due to the amount of extract available. The results of these bioassays are shown in Table 4. Here the typical patterns seen in Tables 2 and 3

Table 3: Results of bioassay tests on Milli-Q extracts of sediments.

Site[a]	Micro-tox[b]	SOS[b] +S9	SOS[b] -S9	*C. dubia*[b]	Nematode[b] % A	Nematode[b] % B	*D. magna*[b]	*Spirillum volutans*[b]	Seed[b] %	Root Length[b]	ATP-TOX[b]	Tot. Pts.
Hny. H.	N.D.[c]	1.0	0.9	N.D.	94	80.3	N.D.	+	100	78.4	2.8	6
St. EOP	N.D.	1.17	0.89	N.D.	86	78.4	EC_{20}	N.D.	100	N.D.	4.9	3
H.H.CP	N.D.	1.23	0.76	N.D.	87	95.6	N.D.	N.D.	84.2	N.D.	22.8	2
H.H. 3.1	N.D.		1.06	N.D.	94	79.6	63%	N.D.	100	N.D.	5	7
H.H. 4.1	N.D.		1.45	N.D.	82	36.4	10%	N.D.	100	N.D.	27.5	22
H.H. 3.3	16.92		1.0	N.D.	34	0.8	0.19%	+	85	54.1	18.2	39
H.H. 4.3	N.D.		1.69	N.D.	96	O	28%	N.D.	85	N.D.	47.9	24
H.H. 3.2	N.D.		1.08	N.D.	97	89.7	72%	N.D.	100	63.9	5.7	8

[a] Abbreviations: Hny H. is Honey Harbour; St. EOP is Stelco end of pier; H.H. is Hamilton Harbour.

[b] Microtox: EC_{50}% mL; SOS induction factor +S9, -S9; *C. dubia*: percent of the sample showing reproduction inhibition; Nematode: A: percent surviving; B: percent maturing; *D. magna*: EC_{50} as a percent of the sample; *Spirilum volutans*: 120 minute test; Seed test: percent germination; Root length percent inhibition; ATP-TOX system percent inhibition.

[c] N.D. is not detected.

Table 4: Results of bioassay tests on 10%-DMSO plus 10% methanol extracts of sediments.

Site[a]	Micro-tox[b]	SOS[b] +S9	SOS[b] -S9	Nematode[b] % A	Nematode[b] % B	*C. dubia*[b]	Tot. Pts.
Hny. H.	N.D.[c]	1.54	1.76	86	67.9	N.D.	9
St. EOP	N.D.	1.35	1.76	91	82.9	N.D.	6
H.H. CP	N.D.	1.43	1.94	89	94.1	N.D.	6
H.H. 3.1	N.D.	N.T.[c]	1.08	86.5	0	N.D.	12
H.H. 4.1	33.88	N.T.	1.40	0	0	1.0%	36
H.H. 3.3	N.D.	N.T.	1.10	0	0	0.1%	37
H.H. 4.3	N.D.	N.T.	1.92	99	0	1.0%	25
H.H. 3.2	N.D.	N.T.	1.07	90.7	9.1	10%	16

[a]Abbreviations: Hny H. is Honey Harbour; St. EOP is Stelco end of pier; H.H. is Hamilton Harbour; CP is Carroll's Point.

[b]Microtox: EC_{50}% mL; SOS induction factor +S9, -S9; Nematode: A: percent surviving; B: percent maturing; C. dubia: Percent of the sample showing reproduction inhibition.

[c]N.D. is Not detected. N.T is not tested.

recur, with H.H. 3.3., 4.1, and 4.3 having the greatest toxicant load, H.H. 3.3 having the highest concentration of bioavailable toxicants or genotoxicants, and Stelco end of pier having the lowest.

The Microtox test was found to be positive in only one solvent extract, H.H. 4.1.

The SOS Chromotest results are very interesting in that the three control samples were positive when tested both with and without S-9 addition. Of the five test sites, only H.H. 4.1 and 4.3, the Ekman dredged surface samples, were positive for the presence of direct acting genotoxicants. These same samples produced similar positive responses in the Milli-Q water extracts.

In the *Ceriodaphnia dubia* chronic toxicity test, four positive (chronic toxicity) responses were noted, H.H. 4.1, 3.3, 4.3, and 3.2 with H.H. 3.3 indicating the presence of the greatest concentration of organically extractable toxicants which are able to produce a chronic toxicity effect.

The nematode test was sensitive to every sample tested. Four of the five test site solvent extracts produced negative (no growth) responses in the maturity part of the test and in two of the extracts H.H. 4.1 and 3.3 a complete

Table 5: Summary of bioassay point scores and ranking of sediments.

Sampling Site	Point Score	Rank
Honey Harbour	18	6
Stelco end of pier	12	8
Hamilton Harbour off Carrolls Point	14	7
Hamilton Harbour 3.1	50	4
Hamilton Harbour 4.1	80	2
Hamilton Harbour 3.3	119	1
Hamilton Harbour 4.3	77	3
Hamilton Harbour 3.2	37	5

kill of all J2 test animals occurred. From these data, it is certainly clear that the nematode test is the most sensitive bioassay with these solvent extracts.

In Table 5, the total point score of each sample shown in Table 2, 3 and 4 are summarized with a ranking of samples from those with the greatest concentration of bioavailable contaminants (toxic and genotoxic), H.H. 3.3, to the sample with the least bioavailable contaminants, Stelco end of pier.

In trying to compare these Table 5 point scores and ranking of sediments based on the bioavailability of toxicants and genotoxicants to sediment structure (Table 1), it can be seen that sediment H.H. 3.3 contained the most sand and least amount of silt. However, careful examination of these sediments and bioassay results indicates that it would be very difficult to relate toxicant load to specific sediment fractions in this part of Hamilton Harbour. Another possibly major factor is that the sediment homogenates reflected different core depths and surface areas.

It has been postulated that the majority of toxicants associated with sediments have limited solubility in water [12, 13], thus it is plausible that the concentrations of toxicants combined with or associated with sediment solids may not correlate well with bioassay results. There is growing support for the belief that bottom dwelling organisms receive most of their exposure to toxicants through contact with pore water [14]. Therefore, pore water with its contained dissolved toxicants load may provide the best estimate of sediment toxicity [15], and toxicant bioavailability.

Based on the above, it would appear that pore water bioassay results shown in Table 2, are probably most indicative of the bioavailable toxicant load in these sediments. However, the solvent extraction procedure appears to provide a better estimate of the water insoluble bound organic pollutants which may have the capability of causing genotoxic effects, such as SOS Chromotest and nematode percent maturity tests (Table 4).

Notation

EC_{50} 50% of the exposed animals die within 48 h
H.H. Hamilton Harbour
MAC maximum allowable concentration
St. EOP Stelco end of pier site

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