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Sediment toxicity testing using large water-sediment ratios: An alternative to water renewal

By:
U. Borgmann & W. Norwood

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Author(s): U. Borgmann and W.P. Norwood

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EC Priority/Issue: This is part of Environment Canada's Action Plan (Conserving Canada's Ecosystems). Environmental assessments frequently require sediment toxicity tests. Maintenance of adequate overlying water quality is sometimes a serious problem when conducting long-term chronic sediment toxicity tests. For example, sediment toxicity testing with sediments collected from lakes near Sudbury, Ontario could not be done using standard static test methods; high sulfide concentrations in these sediments resulted in rapid acidification of overlying water. Many laboratories have dealt with this problem by periodically renewing the overlying water (e.g. 2 times/day), but this results in flushing of all toxic substances leached from the sediment. It also requires an elaborate automatic water renewal system, or labour intensive manual renewal.

Current Status: A new static toxicity test procedure was developed using Imhoff settling cones as test chambers. The large water (1 L) to sediment (15 mL) ratio negates the need to periodically change overlying water. Toxic substances leached from the sediment are not flushed out of the test chamber and the relative contribution of dissolved and solid phase contaminants can be determined. The procedure has been tested successfully with four different species (*Chironomus riparius*, *Hexagenia* sp., *Hyalella azteca* and *Tubifex tubifex*).

Next Steps: The new test method has already been used for studying the effects of atmospheric deposition of metals on sediment toxicity in lakes in the vicinity of the Sudbury smelters. It will be used in planned studies in the collaborative Metals in the Environment Research Network (CNTC), and is now a standard method in our laboratory.

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Sediment toxicity testing using large water–sediment ratios: an alternative to water renewal

U. Borgmann*, W.P. Norwood

National Water Research Institute, Environment Canada, 867 Lakeshore Road, PO Box 5050, Burlington, Ontario, Canada L7R 4A6

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Abstract

Deterioration of overlying water quality during toxicity tests with benthic invertebrates is a serious problem with some sediments. One solution is periodic renewal of overlying water. However, this is either labour intensive or requires construction and maintenance of special equipment. Furthermore, water renewal has the potential for flushing toxic chemicals out of the test chamber and establishes nonequilibrium conditions between the water and sediment. An alternative is testing under static conditions using atypical test vessels (e.g. Imhoff settling cones) with a large water volume (1 l) overlaying a much smaller sediment volume (e.g. 15 ml). This results in dramatic improvement of overlying water quality compared to standard static toxicity tests. Compared to water renewal, the test method is much simpler, all toxic substances leached from the sediment are retained in the test vessel, and contaminant concentrations in water and sediment have more time to equilibrate. Chronic sediment toxicity tests (10–28 days) have been conducted successfully under these conditions with *Chironomus riparius*, *Hexagenia* sp., *Hyalella azteca* and *Tubifex tubifex*.

Keywords: Sediment toxicity tests; Static; Water renewal; *Chironomus*; *Hexagenia*; *Hyalella*; *Tubifex*

1. Introduction

Sediment toxicity tests with benthic invertebrates are now quite common, and several detailed standard test procedures have been published (e.g. ASTM, 1994; USEPA, 1994; EC, 1997). These tests can be performed with or without renewal of overlying water. Static tests have performed well in a number of studies, even with exposure durations of up to 28 days (Borgmann and Norwood, 1993; Bailey et al., 1995). Other researchers have, however, found it necessary to renew overlying water in order to maintain water quality (e.g. Ankley et al., 1993). Periodic renewal of overlying water has the potential of flushing toxic chemicals out of the test chamber, thereby reducing sediment toxicity artificially (Ingersoll and Nelson, 1990; Ankley et al., 1993). Alternatively, overlying water quality could deteriorate to unacceptable levels in static long-term tests (e.g. excessive pH shifts due to chemical reactions between sediment components and overlying water), resulting in excessive mortality even in tests with reference sediments. The latter has generally not been a problem in toxicity tests done by several laboratories at the Canada Centre for Inland Waters in Burlington, Ontario, with sediments from the Great Lakes. However, recent attempts to use the same static test procedures with sediments collected from lakes near Sudbury and North Bay, Ontario, a region containing substantial sulfide minerals, resulted in dramatic deterioration of overlying water quality. Tests with *Hyalella* and *Hexagenia* using beakers with a 4:1 water to sediment ratio (40 ml sediment plus 160 ml water) resulted in pH reductions to around 4 and complete mortality of animals, even in sediments collected from relatively uncontaminated lakes in the vicinity of North Bay. Since the pH of the lakes from which these sediments came was consistently above 6, these tests were not representative of in-situ conditions. We were reluctant to switch to water renewal tests, both because we were concerned with flushing potentially toxic chemicals out of the test chambers and maintaining a system far from equilibrium, and because of the cost and potential mechanical problems of automated flow systems. Below we describe experiments leading to an alternative method: static tests with a large water to sediment ratio.

* Corresponding author. Tel.: +1-905-336-6280; fax: +1-905-336-6430.

E-mail address: uwe.borgmann@cciw.ca (U. Borgmann)

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2. Materials and methods

Methods for culturing and toxicity testing followed Borgmann et al. (1989), Reynoldson et al. (1991, 1995) and Borgmann and Norwood (1997), except for changes in exposure vessels, sediment and water volumes, and number of animals added per test vessel, as described in the text. Tests included growth and survival in *Chironomus riparius* (10-day test), *Hexagenia* sp. (21-day test) and *Hyalella azteca* (28-day test), and survival and reproduction in *Tubifex tubifex* (28-day test). Food consisted of ground Tetra-Min® fish food flakes added at the rate of 2.5, 5.0, 7.5 and 10 mg/week in weeks 1, 2, 3 and 4, respectively, for *Hyalella*, 10 mg/week for *Chironomus*, and 10–15 mg/week for *Hexagenia* and *Tubifex*. The test water was dechlorinated Burlington City tap water, originating from Lake Ontario (see Results and discussion section for detailed chemistry). Cultures and experimental animals were kept in an incubator at 23–25°C with a 16 h light:8 h dark photoperiod, except for tests with *Tubifex* which were conducted in the dark; the same conditions were used for the experiments. All test containers (polycarbonate Imhoff settling cones or glass beakers) received gentle aeration through a glass tube capped with the end of a 250 μl polypropylene pipette tip to provide a small diameter opening. Water was not renewed during toxicity tests, except for the first experiment with beakers when most of the overlying water was replaced manually several times as described below.

The use of a large water to sediment ratio requires a nonstandard toxicity test chamber if a reasonable sediment depth is to be maintained. We selected 1-l polycarbonate Imhoff settling cones, plugged at the bottom with No. 4 silicone rubber stoppers (Fig. 1). Although the sides of the cone are not vertical as in the beaker, they are quite steep making it difficult for animals to cling to this surface for extended periods. Addition of 15 ml of sediments results in a sediment depth of about 2.3 cm and a circular surface 3.1 cm in diameter. By comparison, 40 ml of sediment in a 250-ml beaker have a depth of about 1.3 cm and diameter of 6.3 cm. The surface area is, therefore, reduced roughly four-fold, but a substantial sediment depth is maintained. This results in a water to sediment ratio of 67:1. The water to sediment ratio could be increased further by reducing the sediment volume to 10 ml, if needed. The settling cones were placed on a 150 × 70 cm plywood shelf with five rows of 8.3-cm diameter holes, providing space for 50 cones at a time in a temperature-controlled walk-in incubator.

Test sediments were collected from Lake Erie near Long Point, from Hamilton Harbour, and from 12 lakes near and between Sudbury and North Bay, Ontario (46.05–46.54° N, 79.04–80.97° W). Sediments were collected with a mini ponar or box corer in water depths from 9 to 46 m. Only the top 5 cm of sediment were retained from the box corer sample. All sediments were fine-grained silt/clay mixtures. Organic carbon content was 0.4% in Lake Erie, 3% in Hamilton Harbour, and 3.5–8.8% in Sudbury to North Bay lake sediments. Metal concentrations were very low in Lake Erie (Cu, 25; Ni, 19; Zn, 69 μg/g), moderate in Hamilton Harbour (Cu, 100; Ni, 47; Zn, 1235 μg/g) and reference lakes near North Bay (Cu, 40–70; Ni, 50–100; Zn, 160–460 μg/g), and moderate to high in Sudbury area (Cu, 90–2200; Ni, 150–5800; Zn, 120–1100 μg/g) lake sediments.

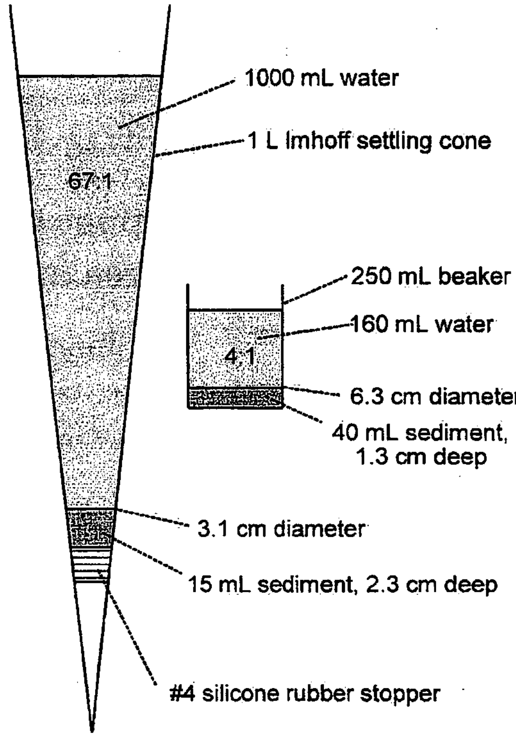

Fig. 1. Diagrammatic representation of a 1-l Imhoff settling cone used as a toxicity test chamber (water to sediment ration of 67:1), and comparison with a 250-ml beaker (water to sediment ratio of 4:1).

Dissolved (filtered through 0.4-μm polycarbonate filters) and total metal concentrations in overlying water during sediment tests were analyzed on a Varian SpectraAA 400 graphite furnace atomic absorption spectrophotometer with Zeeman background correction using a platform and ammonium phosphate modifier for Cd and a partition tube without modifier for Cu and Ni. Dissolved organic carbon and major ions were analyzed by the National Laboratory for Environmental Testing, Environment Canada, Burlington, Ontario.

3. Results and discussion

The first experiment conducted (after the failure of our initial toxicity tests) was a static test in beakers with 4:1 water to sediment ratios (160 ml water + 40 ml sediment), but without test animals present, to accurately quantify the rate of decrease in pH of the overlying water for each sediment, and to determine if the rate of decrease in pH was reduced after several water changes. Fig. 2 shows the pH drop in tests with four of these sediments. The pH dropped to below 5 within 3 days. It returned to around 8 after a 2-week incubation period and three complete changes of the overlying water, but continued to drop after this, although at a reduced rate. Even this reduced rate of pH drop was unacceptable for the purpose of toxicity testing (Fig. 2). Some sediments resulted in a slower pH drop, and some in an even faster pH drop. It is, therefore, not possible to conduct static toxicity tests with these sediments in beakers with a 4:1 water to sediment ratio, even if the overlying water has been renewed several times prior to addition of test animals.

The reason for the rapid drop in pH was not determined, but was likely due to the oxidation of sulfide in the sediment, resulting in the production of sulfuric acid. Sulfate concentrations were elevated in the overlying water in subsequent experiments with these sediments, consistent with this hypothesis. Other anoxic sediments have also been shown to cause dramatic acidification of overlying water following oxidation (de Carvalho et al. 1998). The lakes from which the sediments were obtained were neutral or only mildly acidic (pH in surface water = 6.7–7.5; pH in bottom water = 6.1–7.2). The rapid pH drop in the laboratory was not, therefore, related to low pH in lake water.

The effect of the water to sediment ratio on the rate of drop in pH was tested using the two sediments which resulted in the most rapid drop in pH at a water to sediment ratio of 4:1 (Fig. 3). A substantial pH drop was still observed at water to sediment ratios of 20:1 and 40:1. The pH levelled off after 2 weeks in tests with one of the sediments, but continued to drop in the other sediment. At a sediment to water ratio of 100 or above, however, the pH remained relatively constant during the entire incubation period and was always near or above 8 (Fig. 3). This demonstrates that the pH of the overlying water can be maintained within acceptable limits for toxicity testing if a sufficiently large water to sediment ratio is used. Consequently, we selected a water to sediment ratio of 67:1 (1 l water over 15 ml sediment) for further evaluation, and used Imhoff settling cones instead of beakers to ensure an adequate sediment depth in the test chambers.

The potential effect of the decreased sediment volume and increased density of organisms in the cones on survival and growth of *Hyalella* and *Chironomus* was tested by varying animal density and comparing results to tests in beakers (Table 1). Survival of *Hyalella* was unaffected, even at a density of 30 animals per cone. Growth appeared to be relatively unaffected at densities from 10 to 30 animals per cone, although growth was slightly

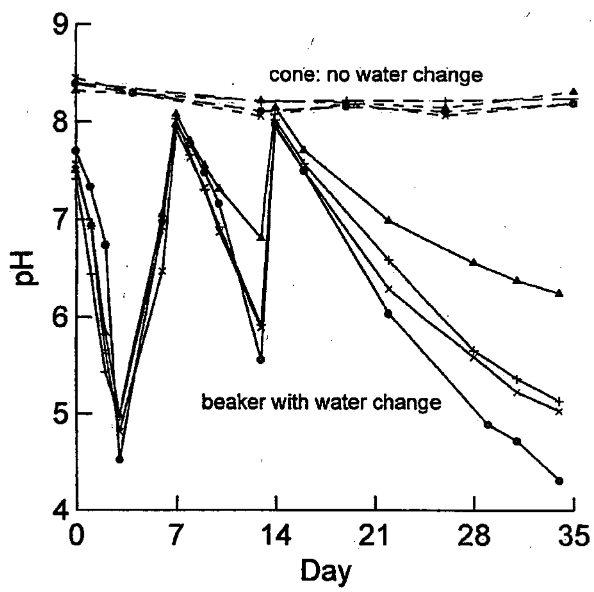

Fig. 2. The change in pH over time in the overlying water in tests with four different sediments. The upper dashed lines represent data from toxicity tests with *Hyalella* conducted in Imhoff settling cones with 15 ml sediment and 1 l of overlying water and no water change during the entire test. The lower solid lines represent data from tests in beakers with 40 ml of the same sediments and 160 ml water and no animals present. The overlying water was changed completely in the beakers after days 3, 6, and 13, resulting in a sharp increase in pH. The sediments were collected from one lake near Sudbury and three lakes near North Bay.

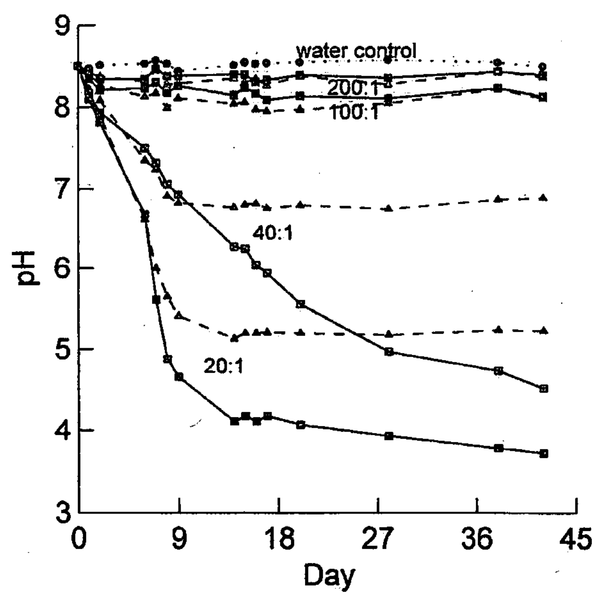

Fig. 3. The change in pH over time in overlying water in tests with two different sediments (solid and dashed lines) using water to sediment ratios ranging from 20:1 to 200:1. The sediments chosen were the ones which resulted in the most rapid drop in pH when tested at a water to sediment ratio of 4:1.

higher at a density of five animals per cone. Surprisingly, even the highest density of 30 animals per cone resulted in better growth than obtained in the beaker (Table 1). Survival and growth of *Chironomus* was also unaffected between densities of five and 15 animals per cone. Again, growth was better in the cones than in the beaker (Table 1). This indicates that growth and survival at densities of 15 *Hyalella* or *Chironomus* per cone with 15 ml of sediment does not result in excessive crowding. The improved growth rates might be the result of a higher density of food (i.e. added food is distributed over a smaller sediment surface) or improved overlying water quality in the cones (i.e. greater dilution of waste products, ammonia, etc.). Conductivity was considerably higher in the beakers than in the cones, indicating a lesser effect of sediment on overlying water quality in the cones (Table 1).

Results of chronic toxicity tests with four species conducted in reference sediments from Hamilton Harbour, Lake Erie, and reference lakes near North Bay indicate that toxicity tests in cones without water changes are feasible, even for sediments which cause rapid deterioration of overlying water quality at lower water to sediment ratios (Table 2). Survival of *Hexagenia* and *Tubifex* was 100% in the cones and growth of *Hexagenia* and reproduction of *Tubifex* were readily measurable and similar to results obtained in other studies (Bailey et al., 1995). By comparison, chronic toxicity tests conducted in beakers with sediments from the same reference lakes near North Bay listed in Table 2 resulted in complete mortality of *Hyalella* and negative growth (i.e. weight loss) in surviving *Hexagenia*. Only 2 *Hexagenia* or *Tubifex* were added per cone because of their larger size; the effect of animal density was not

Table 1
Effect of animal density and container type on survival and growth (SD in parentheses) of *Hyalella* and *Chironomus* in Imhoff settling cones with 15 ml sediment and 1 l overlying water or beakers with 40 ml sediment and 160 ml overlying water, and conductivity at the end of the test (each value is the mean of four replicates)

Test endpoint	Container type–animals per container					
	Cone–5	Cone–10	Cone–15	Cone–20	Cone–30	Beaker–15
Hyalella in Hamilton Harbour sediment, 28-day test						
Survival (%)	95 (10)	95 (6)	97 (4)	93 (5)	89 (4)	93 (6)
Wet weight (mg)	2.08 (0.42)	1.61 (0.53)	1.66 (0.26)	1.76 (0.40)	1.44 (0.50)	0.96 (0.08)
Conductivity (μS/cm)	360 (10)	358 (6)	366 (2)	368 (5)	372 (6)	478 (14)
Chironomus in Lake Erie sediment, 10-day test						
Survival (%)	85 (19)	90 (8)	93 (5)	–	–	83 (12)
Wet weight (mg)	2.48 (0.28)	2.14 (0.31)	2.38 (0.16)	–	–	1.83 (0.12)
Conductivity (μS/cm)	376 (10)	373 (15)	374 (6)	–	–	630 (10)

Table 2
Mean survival, growth, or reproduction in reference sediments from lakes near North Bay, Hamilton Harbour, and Lake Erie for four species in settling cones, based on three (*Hyalella*) or two (all other species) replicate tests per site

Test endpoint	North Bay lakes mean (range)	Hamilton Harbour	Lake Erie
Chironomus 10-day test (15 animals/cone)			
Survival(%)	74 (57–87)	60	70
Final wet weight(mg)	2.28 (1.65–3.36)	2.91	2.69
Hexagenia 21-day test (two animals/cone)			
Survival(%)	100	100	100
Final/initial weight	5.74 (4.74–7.77)	7.31	5.49
Hyalella 28-day test (15 animals/cone)			
Survival(%)	62 (42–84)	87	87
Final wet weight(mg)	1.47 (0.90–1.84)	1.55	1.97
Tubifex 28-day test (two animals/cone)			
Survival(%)	100	100	100
Total young/cone	53.9 (40.5–65)	40	24
Total cocoons/cone	17.3 (12–21.5)	15	7.5

tested with these species. The *Hexagenia* burrow deeply and grow quite large, and visual inspection of the cones suggested that two animals in 15 ml of sediment is probably close to the maximum density desirable.

Use of the Imhoff settling cones with 15 ml of sediment and 1 l of water for chronic toxicity tests resulted in a dramatic improvement in overlying water quality (Fig. 2). The pH was always above 8 at the end of toxicity tests with *Chironomus* or *Hyalella*, and also for *Tubifex* with the exception of a single pH reading of 7.96. The pH dropped lower in tests with *Hexagenia*. Out of 45 tests, 12 containers had a final pH between 7.80 and 7.99, but only two were below 7.80. The concentrations of most of the major ions at the end of chronic toxicity tests in cones were similar to those in the initial test water (Table 3). Alkalinity (i.e. bicarbonate ion) dropped from 1.7 to 1.1 mM on average, in accordance with the drop in pH, and showed the greatest variability in final concentrations (5.4-fold). Most of this variability, however, occurred in tests with *Hexagenia*. Variability was much less (2.2-fold, minimum of 0.9 mM) when the data for *Hexagenia* were excluded (Table 3). Of all the test species used *Hexagenia* appeared to burrow most vigorously and caused the greatest turbidity in the overlying water. This probably resulted in a greater contact between sediment particles and overlying water. A more rapid oxidation of sediment particles (e.g. sulfide) was probably responsible for the greater drop in pH and alkalinity in the tests with *Hexagenia*. The dramatic reduction in concentration range caused by omitting the data for *Hexagenia* was not observed for the other ions. The concentration of sulfate was elevated in some test containers (up to 1 mM), and this was the second most variable ion. The dissolved organic carbon content also increased on average (from 2.0 to 3.2 mg/l) and was quite variable (5.3-fold; Table 3). All of these concentrations are, however, well within the tolerance ranges of the test organisms.

Unlike water renewal systems, static tests with Imhoff settling cones retain toxic substances leached from the sediment. In many cases, this results in relatively stable concentrations in the overlying water after an initial equilibration period. Lead concentrations in overlying water in cones increased rapidly within the first day and peaked within 7–10 days in spiked sediment toxicity tests (Borgmann and Norwood, 1999). Similarly, concentrations of total Ni in water over Richard Lake sediments and Cd over McFarlane lake sediments rose rapidly within the first week, but remained relatively constant after the second week (Fig. 4). Total Cu concentrations, although more variable, demonstrated no clear trend in time. Filtered metal concentrations (data not shown) generally followed total metal trends except for occasional high values probably resulting from contamination during filtering, and filtered Cu values which were consistently below 40 nM within the first week (i.e. the three high values for Cu in Fig. 4 are probably due to Cu in particulates in the sample). Nickel concentrations in water overlying McFarlane lake sediments behaved differently, however. Concentrations rose rapidly within the first week, but then continued to increase more slowly during the remainder of the incubation period (Fig. 4). This caused roughly a doubling in Ni in the water from weeks 2 to 6. It is possible that a

Table 3
Water quality parameters measured in laboratory test (Lake Ontario) water prior to use, in settling cones with 15 ml sediment and 1 l water at the end of chronic tests with *Chironomus* (10 days), *Hexagenia* (21 days), *Hyalella* (28 days), or *Tubifex* (28 days) and in cones without sediment but with animals (cotton gauze added as a substrate)[a]

Parameter	Laboratory test water			In cones with sediment at end of test			
	Mean (*n*)	Min.	Max.	Mean (*n*)	Min.	Max.	Max./min.
Alkalinity (mM)	1.73 (14)	1.62	1.85	1.10 (57)	0.37	2.02	5.4
Omitting *Hexagenia*	–	–	–	1.19 (42)	0.90	1.96	2.2
Cl^- (mM)	0.69 (15)	0.64	0.76	0.69 (55)	0.60	0.75	1.3
SO_4^{2-} (mM)	0.33 (15)	0.28	0.37	0.52 (57)	0.32	1.00	3.1
Ca^{2+} (mM)	0.91 (15)	0.64	0.98	0.87 (57)	0.63	1.39	2.2
Mg^{2+} (mM)	0.36 (15)	0.34	0.37	0.35 (57)	0.27	0.41	1.5
Na^+ (mM)	0.56 (15)	0.49	0.64	0.58 (57)	0.53	0.69	1.3
K^+ (mM)	0.040 (15)	0.033	0.045	0.054 (55)	0.039	0.082	2.1
DOC (mg/l)	2.0 (14)	0.5	4.9	3.2 (57)	1.2	6.3	5.3
	In cones without sediment at end of test			In cones with sediment at end of test			
pH	8.38 (9)	8.03	8.83	8.20 (206)	7.38	8.86	1.48[b]
Omitting *Hexagenia*	8.43 (7)	8.23	8.83	8.29 (161)	7.96	8.86	0.90[b]
Conductivity (μS/cm)	332 (9)	310	365	329 (207)	272	460	1.7
Omitting Hexagenia	327 (7)	310	348	330 (161)	284	460	1.6

[a] Test sediments originated from 12 lakes (21 sites) in the Sudbury to North Bay area of Ontario plus one site each in Hamilton Harbour and Lake Erie

[b] Max.–min.

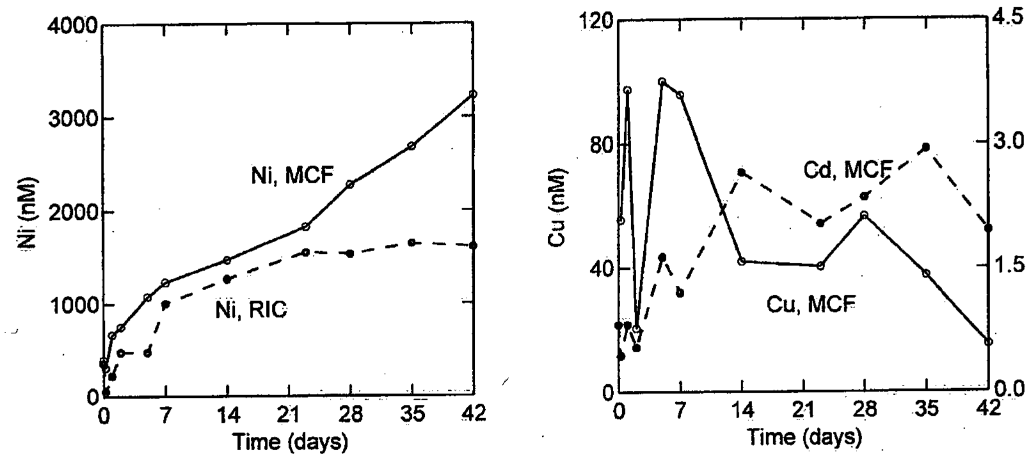

Fig. 4. Total Ni, Cd or Cu concentrations in overlying water in Imhoff settling cones with 15 ml of sediment from McFarlane (MCF) or Richard (RIC) Lake and 1 l of overlying water during the course of a 6-week incubation.

quasi-equilibrium was established within the first week, but that continued aeration of the overlying water allowed a gradual oxidation of the sediment, releasing Ni bound to sulfide in the sediment. A perfect equilibrium is not, therefore, always achieved between the overlying water and the sediment. However, the relative changes in metal concentrations in overlying water after an initial 1–2 week equilibration period are much smaller than the rapid changes occurring within the first day, and the changes likely to occur during periodic water renewal. Furthermore, development of a quasi-equilibrium between overlying water and sediment allows additional toxicity testing with the overlying water (e.g. by placing animals in cages over the sediment) which can be helpful in elucidating the relative importance of dissolved metal versus metal in the solid phase (Borgmann and Norwood, 1999).

The performance of toxicity tests conducted with cones and beakers was compared in Pb-spiked sediment toxicity tests with Hamilton Harbour sediments. The chronic (4-week) LC_{50}s for Pb-spiked sediment were the same in the two test vessels. Trimmed Spearman-Karber LC_{50}s were 35, 37, 36, and 32 μmol/g dry weight (mean μ35 mol/g) in separate experiments with beakers, and 33 and 37 (mean 35) μmol/g in two experiments with cones (Borgmann and Norwood, 1999). Overlying water quality was, however, less affected by the sediment in the cones. Furthermore, total (but not bioavailable) dissolved Pb was lower in the cones than in the beakers, probably because of the lower dissolved organic carbon concentrations and a reduced Pb-complexing capacity in the water. These results provide further evidence that toxicity tests using Imhoff settling cones are feasible and reliable.

4. General discussion

No sediment toxicity test method is perfect. All methods involve handling of the sediment to some degree, and overlying water quality during the test is unlikely to be identical to that observed in the field. Maintenance of adequate overlying water quality is, however, essential for survival of test organisms, and this can be achieved either by periodic water renewal or by using large water to sediment ratios in static tests. For example, one standard method for conducting toxicity tests with *Hyalella* using water renewal recommends water and sediment volumes of 175 and 100 ml, respectively, and a renewal rate of two volume additions/day for 14 days (EC, 1997). This is equivalent to a renewal rate of 52.5 ml of overlying water per 15 ml of sediment per day, or 1 l in 19 days. The total volume of overlying water used per unit volume of sediment would, therefore, be the same in this renewal test and in a static test with cones (15 ml of sediment and 1 l of water) if the test duration was 14 days and an equilibration time of 5 days was used before addition of test organisms. However, the time course of change in concentrations of chemicals leached into overlying water will likely differ in the two test methods. Periodic renewal of overlying water has the potential of flushing out toxic chemicals leached from the sediment (Ankley et al., 1993). If the amount of these chemicals is limited, then there is the potential for a gradual decrease in toxicity of overlying water (and surface porewater) during the course of chronic toxicity tests. On the other hand, use of a static system retains toxic chemicals in overlying water, but the concentrations of these chemicals can increase over time if equilibration between sediment and overlying water is not rapid (Fig. 4). Changes in the concentrations of toxic chemicals in overlying water in static tests can, however, be minimized by allowing a sufficient equilibration time before adding animals to settling cones (e.g. 1–2 weeks: Fig. 4; Borgmann and Norwood, 1999), and can be monitored by measuring toxic substances at both the time of animal addition and at the end of the experiment.

The use of Imhoff settling cones offers several advantages over water renewal systems. Manual water renewal is not required and reliable automated water renewal equipment does not need to be constructed or maintained. This substantially reduces initial set up costs and/or labour while conducting tests. Furthermore, the small sediment volume (15 ml) makes sieving and sorting of animals from the sediment at the end of the test much simpler. The large water volume (1 l) provides ample samples for water quality analysis, and all chemicals leached from the sediment over the entire course of the experiment are retained (although some may be adsorbed to the container walls). Toxicity tests results, and the reasonably rapid equilibration of contaminant concentrations in overlying water seen in several tests (Fig. 4; Borgmann and Norwood, 1999), suggest that contaminant bioavailability in overlying water is probably similar to that in porewaters near the sediment surface where most benthic organisms reside. Contaminant concentrations in overlying water in the cones, therefore, probably provide a useful indication of the bioavailable fraction of many contaminants (e.g. metals) and should be useful in identifying the cause of sediment toxicity (Borgmann and Norwood, 1999). To date, the only disadvantage we have observed with the cones is that their larger size and unusual shape makes soaking and cleaning between tests more difficult than for beakers. The advantages offered by static tests with Imhoff settling cones do not, however, outweigh the usefulness of water renewal test systems for specific applications, such as when testing contaminated sediments from streams which have clean overlying water. In such a case, continuous replacement of overlying water and removal of leached contaminants would more closely replicate in-situ conditions.

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Environment Canada Environnement Canada

Canada

Canada Centre for Inland Waters
P.O. Box 5050
867 Lakeshore Road
Burlington, Ontario
L7R 4A6 Canada

National Hydrology Research Centre
11 Innovation Boulevard
Saskatoon, Saskatchewan
S7N 3H5 Canada

St. Lawrence Centre
105 McGill Street
Montreal, Quebec
H2Y 2E7 Canada

Place Vincent Massey
351 St. Joseph Boulevard
Gatineau, Quebec
K1A 0H3 Canada

Centre canadien des eaux intérieures
Case postale 5050
867, chemin Lakeshore
Burlington (Ontario)
L7R 4A6 Canada

Centre national de recherche en hydrologie
11, boul. Innovation
Saskatoon (Saskatchewan)
S7N 3H5 Canada

Centre Saint-Laurent
105, rue McGill
Montréal (Québec)
H2Y 2E7 Canada

Place Vincent-Massey
351 boul. St-Joseph
Gatineau (Québec)
K1A 0H3 Canada