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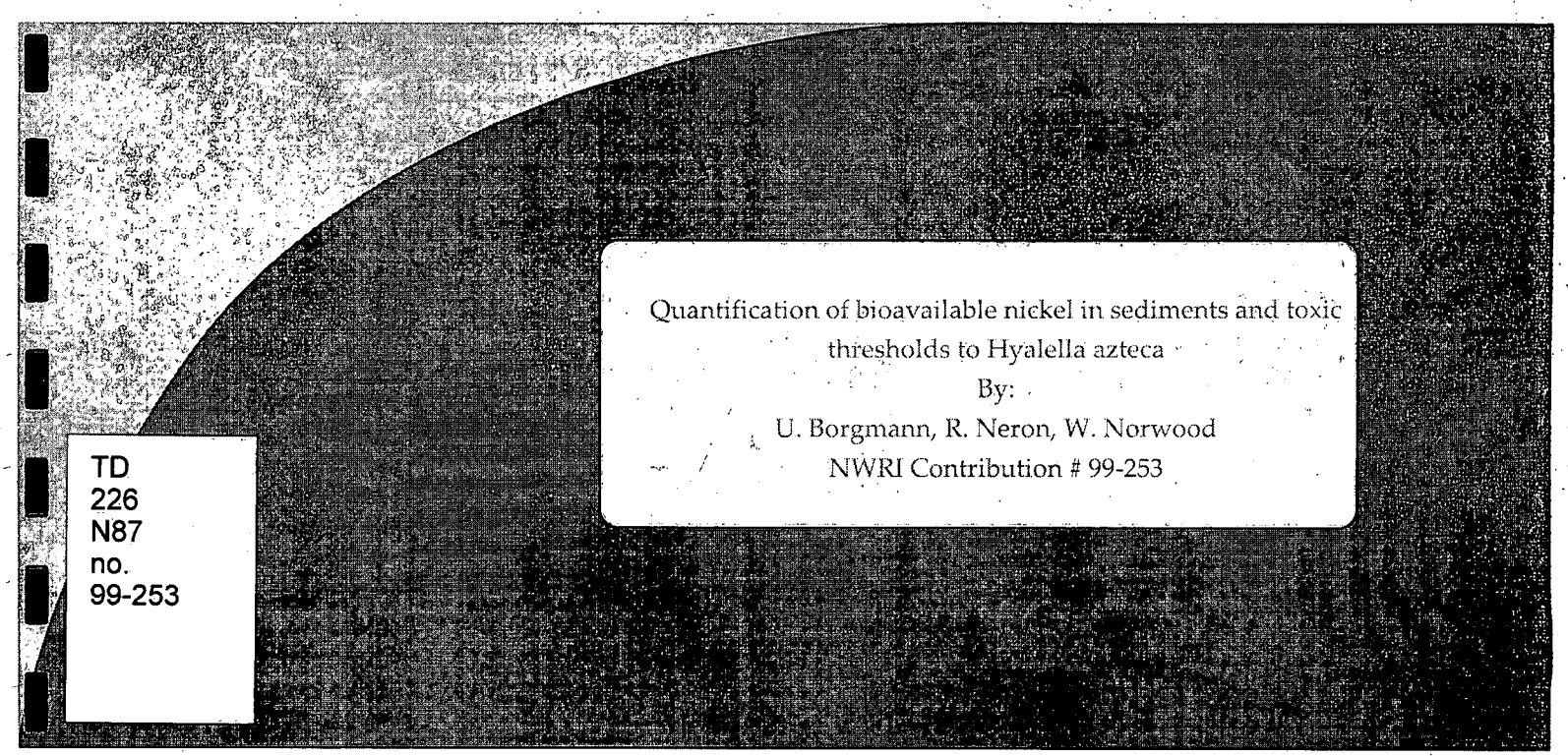

Quantification of bioavailable nickel in sediments and toxic thresholds to Hyalella azteca

By:

U. Borgmann, R. Neron, W. Norwood

NWRI Contribution # 99-253

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MANAGEMENT PERSPECTIVE

Title: Quantification of bioavailable nickel in sediments and toxic thresholds to *Hyalella azteca*

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EC Priority/Issue: This is part of the NATURE business line, Result: Human impacts on the health of ecosystems are understood and reduced, Sub-result: Contribute to the actions to reduce the negative impacts on the health of ecosystems, NWRI project: Sediment Assessment and Restoration. This study addresses the issue of identifying the cause of toxicity in metal contaminated sediments and is directly relevant to Environmental Effects Monitoring for Mining, CEPA assessments of smelter emissions, and other mining and metal contamination priorities. It also relates to issues surrounding Sediment Quality Guidelines, since it demonstrates how metal bioavailability and effects can be predicted even though total metal concentrations in sediments are not reliable predictors of biological effects.

Current Status: This study demonstrates that toxicity of Ni in sediments is a function of Ni accumulated in *Hyalella*, and not total Ni in the sediment. It also shows that bioavailability and toxicity is due to dissolved metal and that metals in overlying water in toxicity tests can also be used to identify cause and effect, but only if tests are conducted in Imhoff settling cones and not in beakers. This study forms the scientific basis for identifying Ni caused toxicity in field studies currently in progress, including the study on smelter effects in the Sudbury area and MITE studies at Rouyn-Noranda and other areas.

Next Steps: Results from this study will be used to quantify Ni bioavailability and effects in current and future field studies. They will also be used in future analyses to derive true cause-and-effect based chemical Sediment Quality Guidelines.

Dosage du nickel biodisponible dans les sédiments et seuils de toxicité pour *Hyalella azteca*

U. Borgmann, R. Néron et W. P. Norwood

SOMMAIRE À L'INTENTION DE LA DIRECTION

Cette étude fait partie du secteur d'activité de la NATURE, résultat : les impacts des humains sur l'état des écosystèmes sont compris et atténués, sous-résultat: contribuer à des mesures visant à réduire les impacts négatifs sur l'état des écosystèmes, projet de l'INRE : évaluation et assainissement des sédiments. Cette étude porte sur la question de la détermination des causes de la toxicité dans les sédiments contaminés par les métaux, et elle est concerne directement la surveillance des effets sur environnement des exploitations minières, les évaluations selon la LCPE des émissions des fours de fusion, ainsi que d'autres priorités relatives aux activités minières et à la contamination par les métaux. De plus, elle touche des questions concernant les lignes directrices relatives à la qualité des sédiments, étant donné qu'elle montre comment on peut prévoir la biodisponibilité des métaux et les effets sur l'environnement même si les concentrations de métaux totaux dans les sédiments ne sont pas des facteurs de prévision fiables pour déterminer les effets biologiques possibles.

Cette étude montre que la toxicité de Ni dans les sédiments dépend de la quantité de Ni accumulée dans *Hyalella*, et non de la concentration de Ni total dans les sédiments. De plus, elle montre que la biodisponibilité et la toxicité sont dues aux métaux dissous et que, pendant les essais de toxicité, on peut également mesurer la concentration de métal dans l'eau sus-jacente pour déterminer les cause et les effets, mais seulement si ces essais sont effectués avec des cônes de décantation d'Imhoff, plutôt qu'avec des béchers. L'étude sert de base scientifique pour l'identification de la toxicité causée par le Ni dans le cadre d'études sur place en cours, notamment l'étude sur les effets des fours de fusion dans la région de Sudbury, ainsi que les études MITE à Rouyn-Noranda et dans d'autres régions.

Les résultats de cette étude serviront à quantifier la biodisponibilité du Ni et ses effets dans le cadre d'études en cours et d'études sur place ultérieures. De plus, ils serviront à des analyses ultérieures pour le développement de lignes directrices relatives à la qualité chimique des sédiments basées sur les vraies causes et effets.

Résumé

La bioaccumulation et la toxicité chronique du nickel pour *Hyalella azteca* dans des sédiments enrichis de nickel dépendaient fortement de la source de sédiments utilisée. La plage totale des CL_{50} en fonction de la concentration des sédiments variait selon un facteur 20. Habituellement, les différences dans la toxicité du nickel correspondaient aux différences dans la bioaccumulation de ce métal, et la toxicité exprimée en concentration dans l'organisme variait d'un facteur inférieur à trois. Donc, les concentrations dans l'organisme permettent d'obtenir des prévisions beaucoup plus fiables de la toxicité du nickel dans les sédiments que les concentrations de ce métal dans les sédiments. De plus, la concentration de nickel dans l'eau sus-jacente était aussi une variable indicatrice fiable de la toxicité du nickel, mais seulement si, pour les essais, on utilisait des cônes de décantation Imhoff avec de grands rapports eau - sédiments (67 : 1). Lors des essais dans des béchers, les CL_{50} pour l'eau sus-jacente variaient d'un facteur 18. On obtenait des concentrations de Ni dans les sédiments et dans l'organisme tolérées par *Hyalella* étaient légèrement supérieures en utilisant les cônes plutôt que les béchers. La reproduction ne semblait pas touchée notablement par le Ni aux concentrations inférieures à la CL_{50} et les valeurs de CE_{50} (10 semaines) des essais de survie et de production de biomasse (notamment ceux de survie, de croissance et de reproduction) n'étaient que très faiblement inférieures à celles des CE_{50} (4 semaines) (survie et croissance seulement).

Mot-clés: nickel, biodisponibilité, toxicité des sédiments, *Hyalella azteca*

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Quantification of bioavailable nickel in sediments and toxic thresholds to *Hyalella azteca*

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"Capsule": *Metal concentrations in the tissues of exposed animals and in water are good indicators of sediment toxicity.*

Abstract

Bioaccumulation and chronic toxicity of nickel (Ni) to *Hyalella azteca* in Ni-spiked sediments was strongly affected by the source of sediment used. The total range in LC50s on a sediment concentration basis ranged over 20 fold. Differences in Ni toxicity generally matched differences in Ni bioaccumulation, and toxicity expressed on a body concentration basis varied less than three fold. Body concentrations, therefore, provide a much more reliable prediction of Ni toxicity in sediments than do concentrations in the sediment. Ni in overlying water was also a reliable predictor of Ni toxicity, but only in tests conducted in Imhoff settling cones with large (67:1) water to sediment ratios. Overlying water LC50s for tests in beakers varied 18 fold. Sediment and body concentrations of Ni tolerated by *Hyalella* were slightly higher in cones than in beakers. Reproduction was not affected significantly by Ni at concentrations below the LC50 and 10-week EC50s for survival and biomass production (including survival, growth and reproduction) were only marginally lower than 4-week EC50s (survival and growth only).

Keywords: Nickel; Bioavailability; Sediment toxicity; *Hyalella azteca*

1. Introduction

It is well recognized that the concentration of total metals in sediments is not a reliable indicator of potential toxic effects because the bioavailability of metals in sediments is quite variable (Luoma, 1989; Ankley et al., 1994, 1996a). A variety of chemical techniques have been proposed in an attempt to quantify the bioavailable fraction of the metal and obtain better estimates of metal-induced biological effects (Tessier and Campbell, 1987; Ankley et al., 1996b). However, the most direct way of quantifying bioavailable contaminants is to measure bioaccumulation by organisms. The critical body residue approach for predicting toxicity of organic contaminants has been promoted by numerous authors (Connolly, 1985; Landrum et al., 1992; McCarty and Mackay, 1993; Connell, 1995; Hickie et al., 1995), and has been applied successfully to contaminated sediments (Driscoll and Landrum, 1997). Body concentrations have also been found to be better predictors of toxicity than water or sediment concentrations for metals (Borgmann et al., 1991, 1998; Borgmann and Norwood, 1997a). This strongly supports the use of bioaccumulation measurements for predicting metal toxicity in sediments. However, critical studies have not been performed in which the relationship between metal bioaccumulation and chronic toxicity to benthic organisms has been compared in several sediments of different chemical composition spiked with metals. If metal bioaccumulation is a reliable predictor of toxicity, then this relationship should be constant and independent of the source of sediment used in spiked sediment toxicity tests.

The purpose of this study was to compare bioaccumulation and toxicity of nickel (Ni) to *Hyalella* in Ni-spiked sediments using several sediments with dissimilar composition. The main objective was to determine if bioaccumulation was indeed a reliable predictor of toxicity in sediments with different metal bioavailability. Secondary objectives were to examine the relationship

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between toxicity and Ni in overlying water, and to compare toxicity tests conducted in beakers with those conducted in Imhoff settling cones. Although bioaccumulation is the most direct indicator of bioavailable metal for most metals, some metals such as copper (Cu) and zinc (Zn) are regulated to varying degrees in animals tissues, and body concentrations may not vary in a linear or predicable fashion with bioavailable metal in water or sediment (Borgmann and Norwood, 1995, 1997b). In this case an alternate measure of bioavailable metal is needed, and metals in overlying water may be useful for predicting sediment toxicity. However, not all metal in overlying water is necessarily bioavailable, and sediments may affect the complexing capacity of the overlying water in toxicity tests (Deaver and Rodgers, 1996; Borgmann and Norwood, 1999a). Toxicity tests conducted in Imhoff settling cones are performed with much larger water volume-to-sediment volume ratios, and consequently have more constant overlying water quality than do tests conducted in beakers (Borgmann and Norwood, 1999b). The greater consistency in water quality (e.g. pH and complexing capacity) should improve predictions of Ni bioavailability and toxicity from measurement of Ni in overlying water. We wanted to test this hypothesis.

2. Materials and methods

Amphipods were cultured in dechlorinated Burlington city tap water (from Lake Ontario, hardness 130 mg l^{-1}, alkalinity 90 mg l^{-1}, pH 7.9–8.6) as described in Borgmann et al. (1989). Young were harvested and culture water was renewed weekly, providing a continuous supply of 0–1-week old amphipods for experiments. Cultures and experimental animals were kept in an incubator at 23°C with a 16 h light:8 h dark photoperiod; the same conditions were used for the experiments.

Sediments used for spiking with Ni were collected from Lake Erie near Long Point (42.56° N 80.04° W), Severn Sound in Georgian Bay, Lake Huron (44.77° N 79.72° W), and near Wasaga Beach, Georgian Bay (42.70° N 80.07° W). These sites were selected from among a database of reference sites collected by T. Reynoldson, National Water Research Institute, because they differed dramatically in composition and represented some of the extremes in Great Lakes sediment composition. The Lake Erie and Severn Sound sediments at these sites are both fine grained (60–74% silt, 29–34% clay, 2–7% sand). However, Lake Erie sediments are rich in calcium (13–15% CaO) and low in total organic carbon (0.2–0.5%) whereas Severn Sound sediments have a lower calcium (3% CaO) and higher organic carbon (7%) content (T. Reynoldson, unpublished data). The Wasaga sediment at this site is mostly (>99%) sand and is low in both calcium (3% CaO) and organic carbon (0.25%). Sediments were spiked with Ni by mixing equal volumes of sediment and 20 mM $NiCl_2$ in Milli-Q de-ionized water in polypropylene bottles and rotating the mixture for 24 h at 4 rpm on a mechanical mixer. The sediments were then allowed to settle and the surface water was decanted. Control sediment was treated in the same way, but without $NiCl_2$ added to the water. Spiked sediments of lower concentration were then made by thoroughly mixing 0.5, 1, 3, 6, 10, 18, 32, or 56% metal-spiked sediment with control sediment. Sediment spiking was performed only once for each sediment type and a single sediment batch was produced for each Ni concentration. The same sediment batches were used throughout to minimize differences between experiments and container types. Measured Ni concentrations (μmol g^{-1} dry mass) in sediments were linearly related to the percent spike, but Severn sediment absorbed and retained the most Ni, while Wasaga sediment absorbed the least for the same level of spiking (Table 1).

Four-week chronic toxicity tests were conducted as described in Borgmann and Norwood (1999a), but without cages and with two different experimental containers: 250-ml glass beakers, and polycarbonate Imhoff settling cones. A volume of 40 or 15 ml sediment and 160 or 1000 ml of overlying water was added to the beakers and cones, respectively, giving a water to sediment ratio of 4:1 for the beakers and 67:1 for the cones. The much larger water-to-sediment ratio in the cones ensures a more stable overlying water quality. This results in improved survival of control animals when testing sediments which alter overlying water chemistry in static tests with smaller water-to-sediment ratios (Borgmann and Norwood, 1999b). The test containers were aerated for 1–2 weeks to equilibrate and allow oxygenation of the surface sediment before addition of animals. Aeration continued throughout the experiments. Measured oxygen concentrations were always

Table 1
Nickel (Ni) concentrations measured in Lake Erie, Severn Sound, and Wasaga Beach spiked sediments

Nominal Ni (percent spiked)	Measured Ni (cdμmol g^{-1} dry wt.)		
	Erie	Severn	Wasaga
0	0.48 (0.08,4)[a]	0.56 (0.13,4)	0.09 (0.05,3)
0.5	0.68 (–,1)	0.80 (–,1)	0.12 (0.08,2)
1	0.93 (–,1)	1.18 (–,1)	0.18 (0.16,2)
3	1.42 (0.19,3)	2.35 (–,1)	0.24 (0.16,3)
6	2.33 (0.71,4)	5.18 (1.14,3)	0.71 (0.27,4)
10	3.33 (0.72,4)	8.08 (1.04,2)	1.43 (0.59,4)
18	3.71 (0.20,3)	13.71 (5.16,2)	1.73 (0.46,2)
32	8.01 (1.25,2)	24.78 (6.45,2)	2.11 (0.77,2)
56	18.27 (–,1)	47.85 (–,1)	6.98 (–,1)
100	23.80 (–,1)	72.68 (–,1)	15.35 (–,1)

[a] (S.D., *n*).

above 6.6 mg l^{-1} and ammonia was always below 0.3 mM, and usually non-detectable. Measured conductivity at the end of the exposure period averaged 771 μS cm^{-1} (range 599–1052) in beakers with Lake Erie sediments, 672 (543–997) in beakers with Severn sediments, and 410 (247–583) in beakers with Wasaga sediments. Conductivity was lower and ranges were smaller in cones (347–435 μS cm^{-1}). For comparison, conductivity in beakers without sediments but with *Hyalella* and cotton gauze as a substrate gave conductivity readings of 346–389 μS cm^{-1} after 28 days. The pH at the end of the exposure period ranged from 8.2 to 8.8 in all test containers with Lake Erie or Wasaga sediments. Cones with Severn sediments had marginally lower pH values (8.0–8.3), but beakers with Severn sediments had pH values between 5.6 and 6.6 at the end of the experiment.

Nine separate sediment toxicity tests were run, including two tests with beakers and one with cones for each sediment type (Table 2). All tests with beakers were completed within 4 months of sediment spiking, but tests with cones were completed 16 months later after development of the cone method. Prolonged storage of sediments could affect contaminant bioavailability, but this was probably not the case in the present study because Ni bioaccumulation from sediments was not significantly different in experiments with beakers and cones (see Section 3.2 below). Twenty 0–1-week-old amphipods were added to each beaker and 15 to each cone. Tetra-Min® fish food flakes were added to each test container in the following amounts: 2.5 mg at the start of week 1, 2.5 mg twice in week 2, 2.5 mg three times in week 3, and 5 mg three times in week 4 and beyond. Feeding did not affect water clarity and all food added was either eaten or decomposed rapidly. Two replicate test containers were used in each experiment, giving 30 (cones) or 40 (beakers) animals per test concentration per experiment, except in the cone experiment with Lake Erie sediments in which three replicates were used. Surviving animals from the third replicate were sacrificed at the end of 4 weeks for body concentration analysis as in all other experiments, but those from the other two replicates were counted and placed into a fresh batch of spiked sediments for an additional 6 weeks to obtain estimates of reproduction. One millilitre-water samples were collected with 0.4 μm filter syringes for analysis of dissolved Ni at the end of the exposure period. The surviving amphipods were sorted from the sediment by sieving and rinsed in clean water. They were placed in 120-ml plastic specimen containers with 50 μM EDTA and cotton gauze for 24 h to clear their guts. For comparison, some of the surviving animals in the beaker experiments were also dried and digested immediately, without gut clearance. Amphipods were weighed as a group to provide a mean mass per container for growth rate comparison, and then dried at 60°C overnight.

For comparison with the sediment toxicity tests, several experiments were also performed to determine the chronic toxicity of Ni in water-only exposures using procedures summarized in Borgmann et al. (1998). Twenty 0–1-week-old *Hyalella* were exposed to Ni in 250 ml of lab water in 500-ml Erlenmeyer flasks with weekly changes of water. Cotton gauze was added as a substrate. Unlike the sediment exposure tests, survivors from water-only experiments were not placed in clean water for 24 h for gut clearance prior to Ni analysis.

Groups of four dried amphipods (approx. 0.5–3 mg total dry mass) were digested with 70% nitric acid at room temperature for 1 week, after which 30% hydrogen peroxide was added and digestion allowed to continue for another 24 h. Each sample was then made up to 0.5–2 ml with Milli-Q de-ionized water. Acid, peroxide and total volumes were 25 μl, 20 μl and 1 ml respectively, for samples of approximately 1 mg dry mass. These were halved or doubled for samples weighing closer to 0.5 or 2 mg, respectively, keeping the

Table 2
Sediment-based toxicity results

Sediment source	Experiment	Control survival (percent)	LC50 (95% CI) (μmol g^{-1} sediment)	LC25	EC25-growth	EC25-biomass
Erie	Beaker 1	88	2.31 (2.04–2.61)	1.81	1.96	1.67
Erie	Beaker 2	94	3.42 (3.18–3.68)	3.18	2.41	2.34
Erie	Cone	88	3.85 (3.54–4.19)	3.44	2.60	2.53
Severn	Beaker 1	43	6.47 (6.47–6.47)	5.79	n.c.[a]	5.69
Severn	Beaker 2	66	6.59 (6.45–6.73)	5.84	4.34	5.45
Severn	Cone	89	9.16 (7.88–10.63)	7.12	3.40	3.01
Wasaga	Beaker 1	98	0.42 (0.39–0.45)	0.30	0.26	0.26
Wasaga	Beaker 2	94	0.64 (0.50–0.81)	0.39	0.27	0.26
Wasaga	Cone	100	1.41 (1.23–1.61)	0.97	0.99	0.78
Range[b]	–	–	22	23	–	22

[a] Not calculated. Growth reduced by <25% at highest concentration with survivors.
[b] Maximum/minimum.

volume ratios the same. Dried sediment samples were analyzed using similar dry mass:acid:peroxide ratio and digestion times, but with larger volumes (20 mg sediments, 500 µl nitric acid, 400 µl peroxide, 10 ml final volume). Water, digested amphipods, and sediments were analyzed for Ni on a Varian SpectraAA 400 graphite furnace atomic absorption spectrophotometer with Zeeman background correction using a partition tube. All analyses were corrected using appropriate blanks with equivalent acid, peroxide, and Milli-Q deionized water but no sample.

Regression analyses between Ni in water or *Hyalella* against Ni in sediment, or between Ni in *Hyalella* against Ni in water, were performed separately for each experiment using Systat 7.0. All metal concentration data were logarithmically transformed before statistical analysis to normalize the data and equalize variances. Metal concentrations in sediment resulting in 50% mortality (LC50) in individual experiments were estimated using the trimmed Spearman–Karber method (Hamilton et al., 1977). Metal concentrations resulting in 25% mortality (LC25) or 25% reduction in growth or biomass production (EC25) were obtained from linear interpolation off plots of survival, mean body mass, or total biomass per test container against the log of measured Ni in sediment. Survival or growth between adjacent Ni concentrations in these plots were averaged if the results were not monotonic. Detailed curve fitting of mortality or growth rates against Ni, as done previously for lead (Pb) exposure (Borgmann and Norwood 1999a), was not done because individual experiments often contained too few partial effect concentrations to obtain an accurate estimate of the slope of the toxicity curve. The LC50s, LC25s and EC25s based on Ni in sediment were combined with linear regression of Ni in water or *Hyalella* against Ni in sediment to obtain equivalent values on a water or body concentration basis.

3. Results

3.1. Sediment-based toxicity

Survival after 4 weeks in Ni-spiked sediments varied greatly between the three different sediments (Fig. 1). Ni toxicity was greatest in Wasaga sediments and lowest in Severn sediments, with more than a 20-fold range in LC50 and EC25 values (Table 2). Survival of *Hyalella* in un-spiked sediments ranged from 88 to 100%, except for poor survival (43–66%) in beakers with Severn sediments (Table 2, Fig. 1). This was probably due to the low pH (5.6–6.6 by the end of the experiments) observed in these beakers. All other experiments, including tests with Severn sediments in cones, had pH values between 8.0 and 8.8 at the end of the exposure

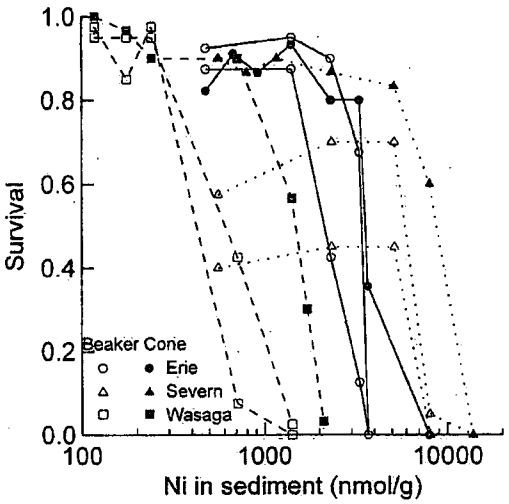

Fig. 1. Survival of *Hyalella azteca* after 4-week exposures to nickel (Ni)-spiked sediments from Lake Erie (solid lines), Severn Sound (dotted lines), or Wasaga Beach (dashed lines) in individual experiments conducted in beakers (open symbols) or Imhoff settling cones (solid symbols) as a function of measured Ni in the sediment.

period. Consequently, the LC50s for Ni in the beakers with Severn sediments represent the toxicity of Ni superimposed on top of a pH stress. Nevertheless, LC50 and EC25 values for these tests were similar to those obtained in the cone experiment, and higher than LC50 and EC25 values for the other test sediments (Table 2). Furthermore, in spite of the high background toxicity in the Severn beakers, survival was still greater at 5-6 µmol Ni/g in Severn sediments than in Lake Erie or Wasaga sediments (Fig. 1). Consequently, Ni toxicity differed substantially in spiked sediment from the three different sites.

3.2. Bioaccumulation and toxic body concentrations

Ni bioavailability, as determined from Ni measured in 24 h gut-cleared *Hyalella*, also varied substantially between the sediment types. Ni bioaccumulation was proportional to Ni in the sediment for any one sediment type ($P < 0.0001$), and was the same for experiments in beakers and cones, but differed between sediment types ($P < 0.0001$; Fig. 2). Analysis of covariance revealed no effect of container type (cone vs. beaker, $P > 0.11$), no significant differences in slope among different sediments ($P > 0.06$) and no interactions between sediment and container types ($P > 0.41$). Not surprisingly, the trend in Ni bioavailability (Wasaga≫Erie > Severn) followed the trend in Ni toxicity (Table 2).

Lethal and effective body concentrations (LBC50s, LBC25s, and EBC25s) for Ni in *Hyalella* were calculated from sediment-based toxicity and Ni bioaccumulated by *Hyalella*. Log(Ni measured in *Hyalella*) was regressed against log(Ni in sediment) separately for each experiment. The sediment-based LC50s, LC25s, and

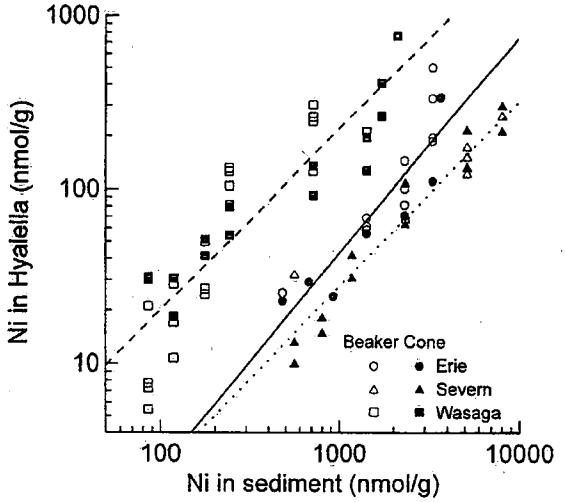

Fig. 2. Nickel (Ni) measured in 24-h gut-cleared *Hyalella* after 4-week exposures to Ni-spiked sediments from Lake Erie, Severn Sound, or Wasaga Beach in beakers or Imhoff settling cones as a function of measured Ni in the sediment. Lines are linear regressions for each sediment type (beaker and cone data pooled).

EC25s for Ni (Table 2) were then converted to body concentration-based values using these regressions (Table 3). This expresses toxicity as a function of the amount of Ni actually accumulated by *Hyalella*, and automatically takes variation in Ni bioavailability into account. Toxicity on a body concentration basis varied much less than on a sediment concentration basis. There was only a 2.3-fold range in the LBC50, compared to a 22-fold range in the LC50 (Tables 2 and 3). For each set of experiments conducted with one sediment type, however, the LBC50 in the cone experiment was always higher than LBC50s obtained with tests in beakers. When LBC50s are compared among beakers or among cones only, the range in LBC50s was less than two fold. Overall, the mean LBC50 was about 1.5-fold higher in the cone (249 nmol g^{-1}) than the beaker (166 nmol g^{-1}) experiments (Table 3). This difference was statistically significant, based on two-way analysis of variance (ANOVA) of log LBC50s against sediment type and test container type ($P<0.01$). It appears that *Hyalella* can tolerate slightly higher body concentrations in the cones, possibly because overlying water quality is superior in the cones and the animals are less stressed by factors other than Ni.

Lethal body concentrations were also calculated from three separate 4-week water-only experiments. The LC50s and LC25s computed from survival and measured Ni in water were combined with linear regressions of log(Ni in *Hyalella*) against log(Ni in water), analogous to the calculations used for the sediment experiments. The resultant LBC50s and LBC25s were slightly higher than those obtained in the sediment exposures because the amphipods were not allowed to clear their guts before they were dried and prepared for analysis (Table 3). Preliminary experiments on Ni uptake rates from water, and efflux following uptake, suggested that instantaneous efflux rates (k_e) are about 0.5 day^{-1}. This results in a loss of about 40% of body Ni in 24 h. By

Table 3
Body concentration-based analysis: coefficients for linear regression of log(nmol Ni g^{-1} in gut-cleared *Hyalella*) against log (μmol Ni g^{-1} in sediment) and toxic endpoints

Sediment source	Experiment[a]	Constant	Slope	R^2	LBC50 (nmol g^{-1})	LBC25	EBC25-growth	EBC25-biomass
Erie	Beaker-1	1.435	2.114	0.886	160	95	113	81
Erie	Beaker-2	1.468	1.462	0.875	177	160	106	102
Erie	Cone	1.542	1.260	0.825	190	165	116	112
Erie	Cone-week 10	1.138	1.949	0.844	–	–	–	–
Severn	Beaker-1	1.494	0.915	0.980	173	156	n.c.	153
Severn	Beaker-2	1.452	1.027	0.943	196	174	128	162
Severn	Cone	1.412	1.141	0.942	323	242	104	91
Wasaga	Beaker-1	2.754	1.500	0.812	153	95	74	76
Wasaga	Beaker-2	2.353	1.049	0.787	140	85	56	56
Wasaga	Cone	2.268	0.887	0.867	251	181	184	150
Range	All	–	–	–	2.3	2.8	–	2.9
Range	Beakers	–	–	–	1.4	2	–	2.9
Range	Cones	–	–	–	1.7	1.5	1.8	1.6
Mean	All	–	–	–	190	143	105	103
Mean	Beakers	–	–	–	166	122	–	98
Mean	Cones	–	–	–	249	194	131	115
Mean[b]	Water-only (not gut cleared)[c]	–	–	–	(330)	(197)	–	–

[a] Four-week exposures except cone-week 10 which is based on Ni measured in *Hyalella* after a water and sediment change and an additional 6 weeks of exposure.

[b] Water-only ($n=3$): LBC50s = 252, 375, 378; LBC25s = 182, 134, 315, respectively.

[c] Water-only correction for gut clearance: LBC50×0.6 = 198; LBC25×0.6 = 118 nmol g^{-1}.

comparison, 24-h gut clearance resulted in a loss of 72% of whole body Ni in sediment-exposed animals (mean 0 h/24 h Ni in *Hyalella* = 3.58, S.D. = 1.10, $n = 17$), presumably because of Ni-contaminated sediment particles in the gut. Gut clearance is critical in bioaccumulation tests with Ni-contaminated sediments, just as it is for Pb (Neumann et al., 1999), but less important in water-only tests. Multiplying the mean LBC50 and LBC25 in the water-only tests by 0.6, to make these comparable to the sediment exposures, gives estimates of the 24-h gut-cleared LBC50 and LBC25 of 198 and 118 nmol g^{-1}, respectively. These values are very close to those obtained in the sediment exposures (Table 3).

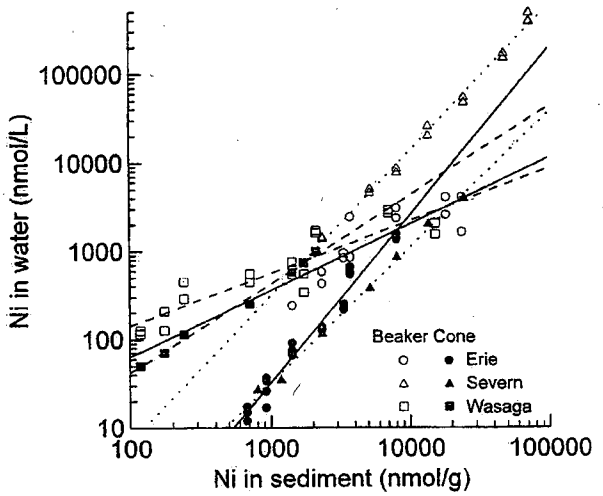

Fig. 3. Nickel (Ni) measured in overlying water after 4-week exposures to Ni-spiked sediments from Lake Erie, Severn Sound, or Wasaga Beach in beakers or Imhoff settling cones as a function of measured Ni in the sediment. Lines are linear regressions for each sediment and container type.

3.3. Significance of Ni in water

Dissolved (0.4-μm filtered) Ni in overlying water was linearly related to Ni in the sediment on a log basis, but the slope and the height of the regression lines varied both between sediment type and test container type (Fig. 3). Experiments with Severn sediments resulted in similar and steep (> 1) slopes with roughly a 10-fold difference in dissolved Ni between beakers and cones. The slope of the regression line in experiments with Lake Erie or Wasaga sediments, however, was steeper for tests done in cones than in tests with beakers (Fig. 3, Table 4). These regression lines were used to convert LC50s and EC25s on a sediment basis to equivalent values estimated on a water concentration basis. Unlike the situation for toxicity on a body concentration basis, toxicity expressed on a water concentration basis varied both between sediment and test container type (Table 4). The overall range in LC50s and EC25s was approximately as great as that seen for sediment-based toxicity (Table 2).

The data for dissolved Ni in overlying water shown in Fig. 3 were obtained from water samples collected at the end of the toxicity test. Previous studies indicated that dissolved Pb in overlying water in beakers and cones, and dissolved Ni, Cd and Cu in cones, usually remains relatively constant over time after an initial equilibration within the first few days (i.e. before test animals are added) although a gradual and continual increase in overlying metal concentration can occur in some situations

Table 4
Water-based analysis: coefficients for linear regression of log(nmol Ni l^{-1} in water) against log (μmol Ni g^{-1} in sediment) and toxic endpoints

Sediment source	Experiment[a]	Constant	Slope	R^2	LC50 (nmol l^{-1})	LC25	EC25-growth	EC25-biomass
Erie	Beaker-1	2.607	0.803	0.784	794	652	696	613
Erie	Beaker-2	2.493	0.695	0.766	731	696	573	562
Erie	Cone	1.533	1.844	0.969	409	333	199	189
Erie	Cone-week 10	1.434	2.064	0.948	–	–	–	–
Severn	Beaker-1	2.483	1.662	0.995	6781	5638	n.c.	5476
Severn	Beaker-2	2.488	1.610	0.995	6396	5271	3262	4715
Severn	Cone	1.503	1.528	0.996	938	638	206	171
Wasaga	Beaker-1	2.778	0.543	0.798	373	314	286	289
Wasaga	Beaker-2	2.714	0.647	0.856	386	283	220	219
Wasaga	Cone	2.622	1.002	0.993	590	408	415	329
Range	All	–	–	–	18	20	–	32
Range	Beakers	–	–	–	18	20	–	25
Range	Cones	–	–	–	2.3	1.9	2.1	1.9
Mean	Cones	–	–	–	610	443	257	220
Mean[b]	Water-only	–	–	–	559	294	–	–

[a] Four-week exposures except cone-week 10 which is based on Ni measured in water after a water and sediment change and an additional 6 weeks of exposure.

[b] Water-only ($n = 3$): LC50s = 462, 578, 655; LC25s = 255, 186, 540, respectively.

(Borgmann and Norwood, 1999a, b). Detailed time series samples for overlying water were not collected in the present study, but unfiltered water samples were collected on the day animals were added and at the end of the experiment to check for major changes in dissolved Ni. Ni in water did not change appreciably in tests with cones at concentrations in the toxic range (final/initial concentration = 1.10, 1.30, and 0.87 for Lake Erie, Severn Sound, and Wassaga sediments, respectively). The same was true for beaker tests with Lake Erie (final/initial concentration = 1.06) and Wassaga (final/initial concentration = 1.44) sediments. The only exception was Ni in water in tests with Severn Sound sediments in beakers. This increased 12 fold between the time animals were added and the end of the experiment. The pH was unusually low in these beakers (5.6–6.6, all other tests were 8.0–8.8). It is likely that a gradual reduction in pH during the test resulted in increased leaching of Ni into the overlying water. In spite of this change in total dissolved Ni, however, the amount of bioavailable Ni did not change. Ni bioaccumulation was the same in beakers and cones with Severn Sound sediments (Fig. 2). Ni bioavailability was, therefore, probably relatively constant throughout the exposure period within each test container in all tests.

The relationship between Ni bioaccumulation by *Hyalella* and Ni in water at the end of the toxicity tests is very different than that between Ni in *Hyalella* and Ni in sediment. In spite of the variation in the Ni in water to Ni in sediment relationships (Fig. 3), all data for Ni in *Hyalella* plotted against Ni in water overlapped for experiments conducted in cones (Fig. 4). No such relationship was observed for the beaker experiments. Ni bioavailability in beakers was much lower than in cones at low concentrations of Ni in water. As Ni in water increased, Ni in *Hyalella* increased rapidly and eventually overlapped Ni values in *Hyalella* in cone experiments for tests with Lake Erie and Wasaga sediments. Ni bioavailability in beakers with Severn sediments, however, was always well below Ni bioavailability in cones. The simple and consistent relationship between Ni in *Hyalella* and Ni in water in experiments with cones suggests that Ni bioavailability is due to dissolved Ni, and not Ni in the solid phase. The highly variable relationship between Ni in *Hyalella* and Ni in water in experiments with beakers, on the other hand, suggests that Ni bioavailability in water is strongly affected by changes in water chemistry, and that sediments can alter overlying water chemistry in test containers with low ratios (e.g. 4:1) of overlying water to sediment.

The differences observed between Ni bioavailability in water in beakers and cones was reflected in Ni toxicity. Although LC50s based on water concentrations showed an 18-fold range in the beaker experiments, there was only a 2.3-fold range in the cone tests. The LC25 and EC25s for dissolved Ni were also relatively constant in the cones (Table 4). Furthermore, LC50s and LC25s based on Ni in overlying water in the cones were also similar to those obtained in 4-week water-only exposures (Table 4). This suggests that Ni in overlying water can be used to quantify Ni bioavailability in sediment toxicity tests, but only in experiments conducted at high water-to-sediment ratios which reduce the impact of sediments on overlying water quality (e.g. cones).

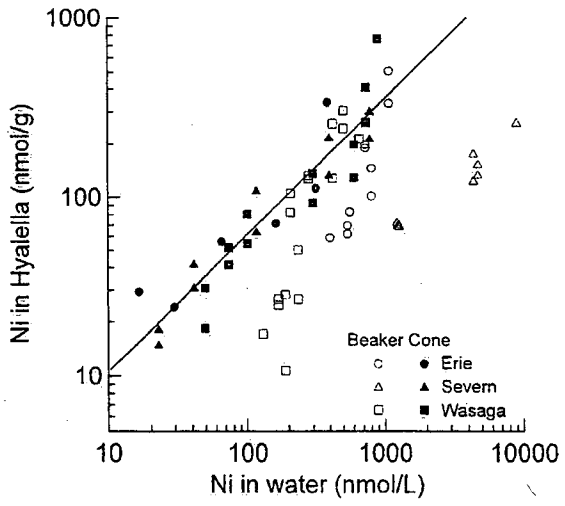

Fig. 4. Nickel (Ni) measured in 24-h gut-cleared *Hyalella* after 4-week exposures to Ni-spiked sediments from Lake Erie, Severn Sound, or Wasaga Beach in beakers or Imhoff settling cones as a function of measured Ni in the overlying water. The line is the linear regression for the pooled data from the cones.

3.4. Effects of Ni on reproduction

The impact of sediment-associated contaminants on reproduction cannot be determined using standard 4-week chronic toxicity tests with *Hyalella* because reproduction begins at 5–6 weeks of age. Ten-week exposures, with weekly water renewal and removal of young, have, however, been used successfully to assess reproductive effects in waterborne toxicity tests (e.g. Borgmann et al., 1993, 1998). The effect of Ni in sediment on reproduction was, therefore, determined in the third cone experiment with Lake Erie sediments by placing survivors from two of the three replicates at each test concentration into cones with a fresh batch of spiked sediments and continuing sediment exposure for an additional 6 weeks. The regressions of Ni in *Hyalella* or Ni in water against Ni in sediment after 10 weeks were similar to the 4-week data (Tables 3 and 4). These were used to convert the sediment-based EC50s to tissue-or water-based EC50s, analogous to procedures used for the 4-week exposures. Unfortunately, it was not possible to reliably identify survivors of the original animals added. The number of adults present at week 10 was often higher than the number of survivors at week 4 due to rapid growth of

the first offspring. Consequently, the data were analyzed in terms of the total number and the total biomass of *Hyalella* present in each cone at week 10 and represent the combined effects of survival, reproduction, and growth (Fig. 5). Some cones contained large numbers of small animals, whereas others contained fewer individuals of larger animals. The biomass data, therefore, showed much less variability between replicates than the data for total number of survivors per cone (Fig. 5). In terms or either number or biomass, EC50 values by week 10 were only slightly lower than 4-week EC50s, regardless of whether toxicity was expressed relative to Ni in sediment, Ni in *Hyalella*, or Ni in water (Table 5). It appears, therefore, that the effects of Ni on growth and reproduction are minimal at concentrations below those which result in chronic mortality. This is similar to results observed for Pb and Zn, but not Cu (Borgmann and Norwood, 1997b, 1999a). Extending toxicity tests with Ni-contaminated sediments from 4 to 10 weeks to include reproduction does not, therefore, dramatically decrease estimated EC50s. However, since reproduction is a more variable endpoint than survival or growth, accurate estimates of subtle reproductive effects (i.e. $\leqslant 25\%$ reduction in young produced) are difficult to determine. It would be necessary to repeat these tests with many more replicates to compare 4- and 10-week EC25s.

4. Discussion

4.1. Quantifying Ni bioavailability and toxicity

As expected, Ni concentrations in the sediment were not a reliable indicator of Ni bioavailability or toxicity in Ni-spiked sediments. Bioaccumulation and toxicity were greatest in sediments off Wasaga Beach, lower in sediments from Lake Erie, and lowest in Severn Sound sediments (Figs. 1 and 2, Table 2). The total range in LC50s was over 20 fold. Differences in Ni toxicity generally matched differences in Ni bioaccumulation, and toxicity expressed on a body concentration basis varied less than 3 fold (Table 3). Body concentrations, therefore, provide a much more reliable prediction of Ni toxicity in sediments than do concentrations in the sediment. The same conclusion was obtained in a study comparing Zn toxicity and accumulation by *Hyalella* exposed to Zn-contaminated sediments (Borgmann and Norwood, 1997a). Toxicity correlated with Zn accumulated by *Hyalella* and not with Zn in the sediment. However, only the reference sediment was spiked with Zn and this was compared to field-collected sediments contaminated with Zn and Cu. The present study is the first in which three different sediments were spiked with a metal (Ni) to show that toxicity can be predicted from metal accumulated by *Hyalella* under conditions in which the toxic agent is definitively known. These results further support the use of body concentrations in benthic organisms for quantifying the toxicity of metals in sediments, rather than total metal concentrations in the sediment.

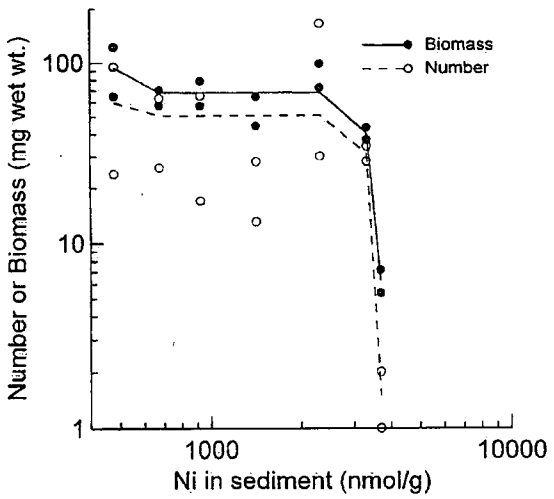

Fig. 5. Total number or total biomass of *Hyalella* in each cone after 10 weeks exposure to nickel (Ni)-spiked Lake Erie sediments as a function of measured Ni in the sediment. Initial number added was 15 animals. Lines represent monotonic average values used to estimate EC50s.

Table 5
Comparison of 4- and 10-week chronic endpoints for toxicity in cones with Ni-spiked sediments from Lake Erie

Parameter	Ni in sediment (μmol g^{-1})	Ni in *Hyalella* (nmol g^{-1})	Ni in water (nmol l^{-1})
Four-week LC50	3.85	190	409
Ten-week EC50, total number	3.34	145	328
Four-week EC50, biomass	3.11	146	277
Ten-week EC50, biomass	3.07	122	274

In addition to Ni bioaccumulation, Ni in overlying water can also be used to predict metal toxicity in sediment toxicity tests, but only if water quality is constant and not affected by the sediment. Water-based LC50s were relatively constant for tests conducted in Imhoff settling cones, but not in tests with beakers (Table 4). The water-based LC50s for Ni were exceptionally high in tests with Severn sediments in beakers. This may have occurred because the sediment released complexing agents into the overlying water which reduced metal bioavailability (Borgmann and Norwood, 1999a) or because Ni toxicity to *Hyalella* decreases at lower pH (Schubauer-Berigan et al., 1993) and pH was reduced in the beakers with Severn sediment. Water quality is less affected by the sediment in cones than in beakers because of the much larger water-to-sediment ratio, and this appears to result in more constant water-based LC50s. This provides a practical alternative to the use of body concentrations for predicting metal toxicity, especially for metals which are regulated in tissues, such as Cu. In

fact, Deaver and Rodgers (1996) demonstrated that Cu toxicity to *Hyalella* in different Cu-spiked sediments was a function of dissolved Cu, although Cu measured by anodic stripping voltammetry correlated better with toxicity than did total dissolved Cu. They used beakers with the same water and sediment volumes used in our study, so the variation in LC50 on a total dissolved Cu basis is not surprising. Toxicity may have been more constant on a total dissolved basis if the tests had been conducted in cones. The bioavailability and toxicity of cadmium (Cd) (Warren et al., 1998) and Pb (Borgmann and Norwood, 1999a) to *Hyalella* are also primarily due to dissolved metal. The concentrations of metals in overlying water may, therefore, be of general use in predicting metal toxicity and identifying the metal responsible for toxicity in sediment toxicity tests with cones.

4.2. Cones versus beakers for sediment toxicity testing

Imhoff settling cones offer several advantages over beakers when conducting sediment toxicity tests. Firstly, the higher water-to-sediment ratio results in more constant overlying water quality in static tests. This increases the likelihood that observed toxicity is due to metals or other contaminants in the sediment and not due to pH changes or other modifying factors. For example, survival in un-spiked Severn sediments was much better in the cones than in the beakers (Fig. 1). Furthermore, within any one sediment type, LC50s on a sediment concentration basis were higher in cones than in beakers (Table 2). In addition, the mean LBC50 value across all sediment types was slightly higher in the cones than in the beakers (Table 3). This suggests that *Hyalella* can tolerate slightly higher metal body burdens in tests conducted in cones, probably because overlying water quality is superior and stress due to factors other than Ni are reduced. The cone tests therefore probably provide a more accurate estimate of the LC50 and LBC50 for Ni in the absence of other stressors.

The second advantage of using cones is that metal concentrations in the overlying water can be used as a secondary parameter for estimating metal bioavailability and toxicity. Although not as direct a measure of bioavailability as metal uptake, metal measurements in overlying water are particularly useful if the metal is regulated in animal tissues (e.g. Cu). Furthermore, metal can be measured in overlying water even if no animals are present in the test chamber. This is useful if no animals survive the exposure, or if a quick estimate of metal bioavailability is desired and test animals are not immediately available. In the latter case, a cone with water and sediment can be set up and aerated for 1–2 weeks following procedures normally used for sediment toxicity testing, but without adding any animals. Measurement of metal in overlying water can then be used to estimate metal bioavailability. This essentially amounts to another chemical extraction method for bioavailable metal, except that the extraction conditions are identical to toxicity test conditions, thereby making the 'extraction' procedure more biologically relevant.

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References

Ankley, G.T., DiToro, D.M., Hansen, D.J., Berry, W.J., 1996a. Assessing the ecological risk of metals in sediments. Environ. Toxicol. Chem. 15, 2053–2055.

Ankley, G.T., DiToro, D.M., Hansen, D.J., Berry, W.J., 1996b. Technical basis and proposal for deriving sediment quality criteria for metals. Environ. Toxicol. Chem. 15, 2056–2066.

Ankley, G.T., Thomas, N.A., Di Toro, D.M., Hansen, D.J., Mahony, J.D., Berry, W.J. et al., 1994. Assessing potential bioavailability of metals in sediments: a proposed approach. Environ. Manage. 18, 331–337.

Borgmann, U., Norwood, W.P., 1995. Kinetics of excess (above background) copper and zinc in *Hyalella azteca* and their relationship to chronic toxicity. Can. J. Fish. Aquat. Sci. 52, 864–874.

Borgmann, U., Norwood, W.P., 1997a. Identification of the toxic agent in metal-contaminated sediments from Manitouwadge Lake, Ontario, using toxicity-accumulation relationships in *Hyalella azteca*. Can. J. Fish. Aquat. Sci. 54, 1055–1063.

Borgmann, U., Norwood, W.P., 1997b. Toxicity and accumulation of zinc and copper in *Hyalella azteca* exposed to metal-spiked sediments. Can. J. Fish. Aquat. Sci. 54, 1046–1054.

Borgmann, U., Norwood, W.P., 1999a. Assessing the toxicity of lead in sediments to *Hyalella azteca*: the significance of bioaccumulation and dissolved metal. Can. J. Fish. Aquat. Sci. 56, 1494–1503.

Borgmann, U., Norwood, W.P., 1999b. Sediment toxicity testing using large water–sediment ratios: an alternative to water renewal. Environmental Pollution 106, 333–339.

Borgmann, U., Ralph, K.M., Norwood, W.P., 1989. Toxicity test procedures for *Hyalella azteca*, and chronic toxicity of cadmium and pentachlorophenol to *H. azteca*, *Gammarus fasciatus*, and *Daphnia magna*. Arch. Environ. Contam. Toxicol. 18, 756–764.

Borgmann, U., Norwood, W.P., Babirad, I.M., 1991. Relationship between chronic toxicity and bioaccumulation of cadmium in *Hyalella azteca*. Can. J. Fish. Aquat. Sci. 48, 1055–1060.

Borgmann, U., Norwood, W.P., Clarke, C., 1993. Accumulation, regulation and toxicity of copper, zinc, lead and mercury in *Hyalella azteca*. Hydrobiologia 259, 79–89.

Borgmann, U., Cheam, V., Norwood, W.P., Lechner, J., 1998. Toxicity and bioaccumulation of thallium in *Hyalella azteca*, with comparison to other metals and prediction of environmental impact. Environmental Pollution 99, 105–114.

Connell, D.W., 1995. Prediction of bioconcentration and related lethal and sublethal effects with aquatic organisms. Mar. Poll. Bull. 31, 201–205.

Connolly, J.P., 1985. Predicting single species toxicity in natural systems. Environ. Toxicol. Chem. 4, 573–582.

Deaver, E., Rodgers Jr., J.H., 1996. Measuring bioavailable copper using anodic stripping voltammetry. Environ. Toxicol. Chem. 15, 1925–1996.

Driscoll, S.K., Landrum, P.F., 1997. A comparison of equilibrium partitioning and critical body residue approaches for predicting

toxicity of sediment-associated fluoranthene to freshwater amphipods. Environ. Toxicol. Chem. 16, 2179–2186.
Hamilton, M.A., Russo, R.C., Thurston, R.V., 1977. Trimmed Spearman–Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11, 714–719.
Hickie, B.E., McCarty, L.S., Dixon, D.G., 1995. A residue based toxicokinetic model for pulse-exposure toxicity in aquatic systems. Environ. Toxicol. Chem. 14, 2187–2197.
Landrum, P.F., Lee II, H., Lydy, M.J., 1992. Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ. Toxicol. Chem. 11, 1709–1725.
Luoma, S.N., 1989. Can we determine the biological availability of sediment-bound trace elements? Hydrobiologia 176/177, 379–396.
McCarty, L.S., Mackay, D., 1993. Enhancing ecotoxicological modeling and assessment: body residues and modes of toxic action. Environ. Sci. Technol. 27, 1719–1728.
Neumann, P.T.M., Borgmann, U., Norwood, W., 1999. Effect of gut clearance on metal body concentrations in *Hyalella azteca*. Environ. Toxicol. Chem. 18, 976–984.
Schubauer-Berigan, M.K., Dierkes, J.R., Monson, P.D., Ankley, G.T., 1993. pH-dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Lumbriculus variegatus*. Environ. Toxicol. Chem. 12, 1261–1266.
Tessier, A., Campbell, P.G.C., 1987. Partitioning of trace metals in sediments: relationships with bioavailability. Hydrobiologia 149, 43–52.
Warren, L.A., Tessier, A., Hare, L., 1998. Modelling cadmium accumulation by benthic invertebrates in situ: the relative contributions of sediment and overlying water reservoirs to organism cadmium concentrations. Limnol. Oceanogr. 43, 1442–1454.

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