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Methods for assessing the toxicological significance of metals in aquatic ecosystems: bio-accumulation–toxicity relationships, water concentrations and sediment spiking approaches☆

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Abstract

Although the published literature abounds with studies showing contamination of aquatic environments by metals, there are very few data which actually demonstrate the biological impact of this contamination. Biological impacts such as alteration of in situ communities and demonstration of toxicity in environmental samples often occur at sites with elevated metal concentrations, but this does not prove that metals are actually responsible for these effects. Correlation is not proof of cause and effect. Metal-induced biological effects cannot usually be inferred from measured environmental concentrations because metal bio-availability can vary dramatically from site to site. Differences in metal bio-availability lead to differences in metal bio-accumulation, which in turn lead to differences in metal-induced effects. On the other hand, metal concentrations in biota are often much better indicators of potential biological impact than concentrations in the environment, because differences in metal bio-availability are automatically taken into account. Measurement of the body concentration of metals is a powerful tool for predicting metal effects, especially for non-essential and non-regulated metals. The body burden approach is more limited when applied to essential metals such as copper and zinc. Alternate methods which provide useful information on metal bio-availability, especially for copper and zinc, include measurement of metals in the overlying water during sediment toxicity tests, and sediment spiking with additional metal. Canadian Crown Copyright © 2000 Published by Elsevier Science Ltd and AEHMS. All rights reserved.

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1. Introduction

Although an extensive literature now exists on metals in the aquatic environment (e.g. Dallinger and Rainbow, 1993; Tessier and Turner, 1995), our understanding of the biological effects of these metals under natural conditions is very limited. There are many publications both on metal concentrations in water, sediments and biota, and on the toxicity of metals as measured in the laboratory. Furthermore, an increasing number of studies have demonstrated environmental effects such as sediment toxicity or

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the absence of sensitive species in areas with high metal concentrations (e.g. Krantzberg, 1994). This has led to the establishment of sediment quality criteria based on correlations between observed effects in the field and measured metal concentrations (e.g. Smith et al., 1996). In spite of such progress, however, it is still extremely difficult to assess accurately the toxicological significance of metals present at any given site, and to attribute toxic effects to metals. Correlation between effects and metal concentrations is not proof that these effects are caused by the metals. This was, in fact, made quite clear in a report on the derivation of sediment quality assessment values by Environment Canada. These guidelines cannot be used to infer cause and effect (Smith et al., 1996). Metal-induced toxicity in sediments has been identified in several studies using toxicity identification and evaluation procedures (TIEs) and related methods (e.g. Ankley and Schubauer-Berigan, 1995; Borgmann and Norwood, 1997b), but such studies are relatively rare within the metal literature. Consequently, our knowledge of the actual biological effects of metals in the environment is still quite limited.

The major problem in understanding metal effects on aquatic biota is the high variation in metal bioavailability in the environment. The concentrations of bio-available metals are not directly proportional to total metal concentrations in water or sediment, and total metal concentrations cannot be used to infer effects. There are two kinds of factors which affect metal bio-availability to aquatic biota: chemical and physical factors acting outside the organism, and biological factors acting within or on the surface of organisms. The former affect most biota in the same way, but the latter effects can be very species specific. Chemical and physical factors affecting metal bioavailability include complexation of metal ions by inorganic and organic complexing agents, adsorption to particulate matter, precipitation and binding within insoluble matrices. Such effects can often be modeled using chemical speciation models such as the free ion activity model. Several examples exist in which speciation modeling, or direct measurement of free metal ions, has been extremely successful in explaining the variations in metal bio-availability and in predicting toxic effects (Campbell, 1995). However, other factors which act within the organism or on the organism's surface can also affect metal bioavailability. This includes, for example, competition between metal and essential ions, or metal and hydrogen ions, on the surface and within the uptake channels for ions in the animal (see review by Campbell, 1995). Since this involves the relative binding strength of various ions to the biota themselves, the magnitude of these effects can vary from one species to another. Such effects are often overlooked and many researchers erroneously assume that toxicity is simply proportional to free ion concentrations. Another example is the presence of organic complexing agents which produce metal complexes which are also bio-available. For example, lipophilic complexing agents can actually facilitate the uptake of metals by allowing direct diffusion through the lipid membranes of organisms. Such metal complexes can be extremely toxic (e.g. Ahsanullah and Florence, 1984) resulting in biological effects at much lower free metal ion concentrations than in their absence. Another biological factor to consider in metal bioavailability is metal uptake through ingestion. This can also be viewed as a chemical speciation problem, but in this case the speciation occurs within the gut of the animal, thereby becoming an organism-specific problem. For example, a more complex digestive physiology generally results in a much higher percentage assimilation of ingested silver and cadmium in bivalves than in zooplankton (Fisher and Reinfelder, 1995). All these factors make prediction of metal effects from chemical measurements difficult.

An example of the difficulty in predicting toxic effects of metals is demonstrated by the acute toxicity of copper to *Daphnia magna* (Fig. 1). The presence of complexing agents, including Tris (tris(hydroxymethyl)aminomethane) and various amino acids, greatly increases the total concentration of Cu tolerated relative to the inorganic medium. However, the concentration of free Cu ion in the medium at the point where 50% mortality occurs decreases to varying degrees as the total concentration of Cu tolerated increases. This indicates that some toxicity is associated with these organic complexes (Borgmann and Ralph, 1983). The same phenomenon was observed when comparing Cu toxicity in artificial media with that in natural waters of similar ionic composition, indicating that some natural complexing

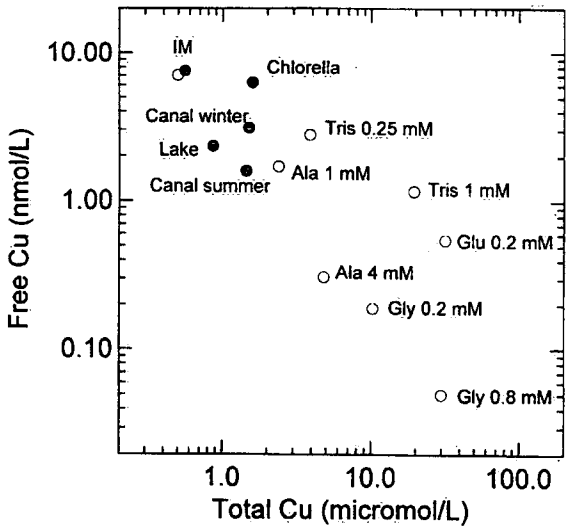

Fig. 1. Relationship between the concentration of free copper ion, as measured using a cupric ion electrode, and total copper resulting in 50% mortality of *Daphnia magna* in 48 h in two separate studies (open symbols, Borgmann and Ralph, 1983; closed symbols, Borgmann and Charlton, 1984) using inorganic medium with no additions (IM), or with added 0.25 or 1 mM tris(hydroxymethyl)aminomethane (Tris), 1 or 4 mM β-alanine (Ala), 0.2 or 0.8 mM glycine (Gly), 0.2 mM glutamate, or *Chlorella* algal cells, or using natural lake water taken from Lake Ontario (Lake) or the Burlington canal in winter or summer.

agents have the same effect (Borgmann and Charlton, 1984; Fig. 1). Only adsorption to *Chlorella* sp. cells appeared to increase the tolerance to total Cu without markedly reducing the amount of free Cu present under lethal conditions (Fig. 1). These effects can be very species specific, since growth reduction of copepods under similar conditions and in the presence of various amounts of Tris was completely predictable from free Cu ion concentrations (Borgmann and Ralph, 1984). This demonstrates the difficulty in predicting toxic effects from chemical measurements alone, and the dangers in extrapolating from one species to another. Similar problems have been encountered in predicting Cu toxicity to fish from free Cu ion concentrations (Erickson et al., 1996). These examples deal only with dissolved Cu. In metal contaminated sediments the solid phase could also contribute to toxicity, and adsorption and precipitation chemistry are much more complex, making prediction of toxicity from chemical measurements even more difficult.

2. Bio-accumulation–toxicity relationships

2.1. Body concentrations as measures of metal bio-availability and effects

One of the best methods of circumventing problems in predicting toxic metal effects from chemical measurements is to measure metal concentrations in the animals themselves, rather than in the water or sediments in which these organisms live. Intuitively, this makes sense because toxic chemicals must usually enter the organism before the toxic effect is expressed. Factors controlling metal bio-availability affect the rate of metal uptake, and this, in turn, is expected to affect metal toxicity. Factors which reduce metal uptake (e.g. increased hardness and complexing ability), generally also reduce metal toxicity. Unfortunately, metal uptake and toxicity are often not reported together in the same study. Laboratory toxicity tests usually relate effects to metal concentrations in water or sediments, and not to concentrations in the biota themselves. Field studies report metal concentrations in water, sediments and biota, but the potential effects of metals in water and sediment cannot be interpreted because of uncertainties in metal bio-availability, and the significance of metal concentrations in biota cannot be interpreted because toxicity–bio-accumulation relationships are often not reported in laboratory studies.

Although relatively rare, a few studies do support the hypothesis that toxicity can be predicted much more accurately from metal concentrations in aquatic biota than in their environment. For example, the chronic toxicity of Cd to the amphipod *Hyalella azteca* in Lake Ontario water, manipulated by addition of complexing agents or sediments, varied over 5000-fold when expressed as nominal Cd added to the water, over 30-fold when expressed as Cd measured in the overlying water, but only 2.6-fold when expressed as Cd measured in the body (Borgmann et al., 1991). Similarly, growth reduction in *Hyalella* occurred at 4-fold lower thallium concentrations in an artificial medium without potassium than in Lake Ontario water, but the body concentration resulting in a 25% reduction in final size was not significantly different between the two media (Borgmann et al., 1998). The toxicity of lead to *Hyalella* in experiments with Pb-spiked sediments, shown to be due to dissolved Pb,

also varied much more on a water concentration basis than on a body concentration basis (Borgmann and Norwood, 1999). The toxicity of Cu to the duckweed *Lemna trisulca* ranged from 3.2 to 55 μmol l^{-1} with increasing EDTA, but the internal tissue Cu associated with 50% reduction in growth only ranged from 21 to 26 μmol g^{-1} (1.3–1.6 mg g^{-1}). In this case toxicity was not proportional to free Cu ion concentrations, estimated to range from 0.01 to 7.9 nM at 50% growth reduction (Huebert et al., 1993). These are all examples which demonstrate that the body or tissue concentration of metal is relatively constant in equally toxic media, even though the metal concentration in the external medium varies considerably.

Whereas the above examples compare toxicity among different media using the same test species, there are also examples where body concentrations help explain wide differences in metal toxicity between species or between toxicants. For example, the 10-day LC_{50} for tributyltin toxicity to marine amphipods varied from 5.2–5.9 nmol l^{-1} in *Eohaustorius washingtonianus* to 79–111 nmol l^{-1} in *Rhepoxynius abronius*, whereas the body concentrations at the LC_{50} overlapped at 139–242 nmol g^{-1} (Meador et al., 1993). Another example is the toxicity of both inorganic and organic mercury to barnacle and brine shrimp larvae (Corner and Rigler, 1958; Table 1). Both toxicants were 100 or more times more toxic to barnacles based on water concentrations, but on the basis of body concentration the toxicity was virtually identical. It is also noteworthy that the toxicity of the organic Hg compound was 20–1000 times more toxic than inorganic Hg on a water concentration basis, but almost equally toxic on a body concentration basis (Table 1). Similarly, the chronic toxicities of Cd, Hg, Tl and Pb to *Hyalella azteca* varied over 13-fold on a water concentration basis, but were relatively constant on a body concentration basis (Borgmann et al., 1998). This does not imply that all four metals, or organic and inorganic forms of Hg, have the same mode of action. However, these results do demonstrate that the high variability seen in toxicity of metals and organo-metals, expressed as concentration in the animal's environment, is often due to differences in bio-availability causing differences in uptake, rather than large inherent differences in toxicity of the chemical inside the body.

Table 1
Three-hour LC_{50} for mercury compounds in *Eliminius* (barnacle) and *Artemia* (brine shrimp) larvae based on water (μmol l^{-1}) and body (μmol g^{-1}) concentrations. Data from Corner and Rigler (1958)

	Water concentration	Body concentration
Mercuric chloride		
Eliminius	1	4.6
Artemia	5000	2.3
n-Amylmercuric chloride		
Eliminius	0.05	3.5
Artemia	5.00	1.4

2.2. Application of bio-accumulation–toxicity relationships to environmental assessment

An example of the application of bio-accumulation–toxicity relationships in identifying the cause of sediment toxicity was demonstrated for Manitouwadge

Table 2
One-week survival and metal uptake in *Hyalella azteca* exposed to Loken (L1–L3) and Manitouwadge (M1–M3) Lake sediments, and 1-week lethal body concentrations resulting in 50% mortality in metal-spiked sediment toxicity tests (LBC_{50}). Metal values expressed as μmol g^{-1}. Distance from mine tailings increases from M1 to M2 to M3. Survival marked with * is significantly reduced ($P < 0.01$). Data from Borgmann and Norwood (1997a,b)

Site	Percent survival	Metal in sediment		Metal in *Hyalella*	
		Zn	Cu	Zn	Cu
L1–L3	76–94	< 2	< 0.4	< 0.96	< 1.24
M1	71	139	31.9	4.59	2.85
M2	41*	64	7.1	8.36	2.31
M3	93	49	10.1	4.31	3.00
1-week LBC_{50}, spiked sediments				8.27	5.41

Lake, which receives drainage from mine tailings (Borgmann and Norwood, 1997b). Copper and Zn concentrations in sediments were much higher in Manitouwadge Lake than in the reference (Loken) lake. The concentrations of both generally decreased with increasing distance from the tailings (Table 2). Significant sediment toxicity was observed, especially at site M2, but the cause of toxicity could not be identified from metal concentrations in the sediment. There was no correlation between metal concentrations in the sediment and toxic effects. On the other hand, bio-accumulation of Zn, but not Cu, closely correlated with toxicity. This suggested that Zn was more likely to be the toxic agent. However, toxicity could also have been due to some other contaminant which correlated with Zn bio-availability. This is a case where good data on the bio-accumulation–toxicity relationship for Cu and Zn are needed. Fortunately, the lethal body concentrations (LBC_{50}) have been determined in experiments with Cu- and Zn-spiked sediments (Borgmann and Norwood, 1997a). The LBC_{50} for Zn (8.3 μmol g^{-1}) matched the amount of Zn accumulated from those Manitouwadge Lake sediments which resulted in roughly 50% mortality relative to the reference sites. The amount of Zn accumulated from M2 sediments was, therefore, sufficient to account for all the observed toxicity. This was not the case for Cu (Table 2). Hence, Zn was probably the toxic agent.

Another example of the use of bio-accumulation–toxicity relationships in assessment of the biological significance of environmental metal concentrations is the evaluation of the relative impacts of Cd and Tl in Lake Ontario and Hamilton Harbour sediments (Borgmann et al., 1998). Accumulation of these metals by *H. azteca* was not proportional to the total amount of metal in the sediments. Concentration factors (concentration in *Hyalella*/concentration in sediment) ranged from 0.08 to 1.04 for Cd and from 0.47 to 1.44 for Tl. Although total metal in the sediments of Hamilton Harbour was higher than in near-shore Lake Ontario sediments, there was more bio-available metal in the lake, and the highest bio-availability was observed in deep-water Lake Ontario sediments, not in the Harbour. Maximum bio-accumulation of Cd (16 nmol g^{-1}) was about 3-fold higher than for Tl (4.9 nmol g^{-1}). Since the body concentration resulting in 25% mortality after 4 weeks was similar for the two metals (270–290 nmol g^{-1}), this implies roughly a 3-fold greater risk from Cd than from Tl. Nevertheless, the bio-availability for both metals was more than 15 times below the critical body concentration, indicating a low risk of effects (Borgmann et al., 1998). In this study, metal uptake by *Hyalella*, coupled with data on the bio-accumulation–toxicity relationships for Cd and Tl, allowed assessment of the relative risk of metal effects, both between sites and between metals.

2.3. Limitations on the use of bio-accumulation–toxicity relationships

As with all scientific methods, no matter how valuable and effective, there are conditions under which the above approach is not appropriate. Body concentrations of contaminants can provide useful information on possible effects only if a strong and clear relationship exists between bio-accumulation and toxic effects. This means that the toxicant cannot be metabolized (generally not a problem with elemental metals) or sequestered in a non-toxic form. For example, barnacles can accumulate large amounts of Zn which is stored in insoluble form in granules within the body (Rainbow and White, 1989). This makes it very difficult to infer effects from total metal in the tissue of these animals. It is also necessary that the metal is not regulated over the concentration range of interest. For example, Cu uptake by *Hyalella* is strongly related to metal in the water in short-term (1–4 weeks) exposures, but regulation of body Cu gradually sets in, and there is no relationship between body-Cu and Cu in the water after 10 weeks (Borgmann et al., 1993; Borgmann and Norwood, 1995). Consequently, body concentrations can be useful indicators of bio-available Cu in water during laboratory tests when cultured amphipods are exposed to environmental samples, but interpretation of the significance of Cu in wild animals, where exposure history is unknown, is much more difficult. Similarly, body concentrations of Cu in *Hyalella* can be used to infer effects from Cu contaminated sediments at concentrations high enough to cause toxicity within 1 week, but not at the lower concentrations resulting in chronic toxicity (Borgmann and Norwood, 1997a,b).

Another factor which must be considered is the

source of the metal and its effect on bio-accumulation and toxicity. For example, the internal distribution of Hg in mayfly larvae is strongly affected by whether uptake is from water alone or from sediment (Saouter et al., 1993). Metals obtained via the gut can result in high concentrations in the gut tissues, but these are not necessarily transferred efficiently to the rest of the body (e.g. Craig et al., 1998). It may be necessary to measure bio-accumulation–toxicity relationships separately for water only and contaminated sediment exposures (e.g. Borgmann and Norwood, 1997a). Problems with variation in internal metal distribution resulting from different modes of metal uptake can often be overcome, but this necessitates careful laboratory studies to define clearly the relationship between metal accumulation and toxicity.

Finally, it must be remembered that the bio-accumulation–toxicity approach, as discussed above, can predict biological effects on the test species, but not necessarily on the ecosystem as a whole. Contaminants which are bio-magnified up the food chain (Hg, polychlorinated biphenyls, DDT) will have their greatest effect at higher trophic levels where body concentrations are greatest. A bio-accumulation–toxicity approach can still be used for such contaminants, but the critical body concentration for ecosystem protection is not the threshold concentration which causes a direct effect on the test organism, but rather that which, once bio-magnified up the food chain, will result in effects in top predators.

3. Alternative approaches to the use of bio-accumulation–toxicity relationships

In those cases where the bio-accumulation–toxicity relationship cannot be used for assessment of potential metal effects, such as when the metal of interest is regulated by the test organism, an alternative is required. Two approaches, analysis of metal concentrations in the water and spiking sediments with additional metal, are discussed below.

3.1. Identification of toxic metals using dissolved metal concentrations in water

The identity of metals responsible for toxicity can sometimes be inferred from dissolved metal concentrations in the overlying water during toxicity tests. Even when metal exposure is caused by metal contaminated sediments, benthic organisms often acquire much of their metal burdens from the overlying water rather than the sediment directly. For example, both bio-accumulation and toxicity of Pb in Pb-spiked sediments was the same for *Hyalella* exposed directly to sediments and for those exposed only to overlying water in cages placed above the sediments (Borgmann and Norwood, 1999). Bio-accumulation and toxicity were, therefore, solely a function of dissolved metal. Consequently, it is sometimes possible to identify the toxic agent from measurements of dissolved metals in sediment bioassays and comparison to metal concentrations in water-only toxicity tests. An example is given in Table 3, which lists Zn and Cu concentrations in the overlying water measured during the toxicity tests described in Table 2. Dissolved Zn concentrations correlated well with toxicity to *Hyalella*, whereas dissolved Cu concentrations did not. Furthermore, dissolved Zn concentrations exceeded the 1 week LC_{50} measured in waterborne toxicity tests, but dissolved Cu concentrations did not. This supports the conclusion that Zn was probably the toxic agent rather than Cu.

One disadvantage of the use of dissolved water concentrations is that a direct correlation between total metal in the water and toxicity cannot always be made. Water chemistry characteristics can affect the bio-availability and toxicity of metals in the water. Measurement of total dissolved metal in the water does not take variations in bio-availability into

Table 3
One-week survival of *Hyalella azteca* exposed to Loken and Manitouwadge Lake sediments and metal in overlying water, and 1-week lethal water concentrations resulting in 50% mortality in water-only toxicity tests (LC_{50}). Data from Borgmann and Norwood (1997b) and Borgmann et al. (1998)

Site	% Survival	Metal in overlying water (μmol l^{-1})	
		Zn	Cu
L1–L3	76–94	< 0.05	< 0.03
M1	71	14	0.30
M2	41*	24	0.09
M3	93	7.1	0.08
1-week LC_{50}, water-only experiments		6.2	2.3

account in the same way that measurement of body concentrations does. In the above example, the same water source was used in the water-only toxicity tests to determine the LC_{50} and for overlying water in the tests with Manitouwadge Lake sediments. However, dissolution of organic matter from the sediment probably reduced both the bio-availability and toxicity of the Zn. Zinc concentrations overlying the sediments from site M1 exceeded the 1 week LC_{50} by more than 2-fold, but toxicity was minimal. This effect is demonstrated further in Table 4. Zinc concentrations in water overlying un-spiked Hamilton Harbour sediments in toxicity tests (1.83 $\mu mol\ l^{-1}$) exceeded the 4 week LC_{25} for Zn in water-only tests (1.70 $\mu mol\ l^{-1}$), suggesting possible chronic toxicity of Zn to *Hyalella* exposed to Hamilton Harbour sediments. However, spiking of Hamilton Harbour sediments with a range of Zn concentrations resulted in 25% mortality in 4 weeks only after overlying water concentrations reached 3.29 $\mu mol\ l^{-1}$ (at 54 $\mu mol\ g^{-1}$ sediment). The reduced Zn toxicity on a water-concentration basis (almost 2-fold) probably resulted from binding of Zn to organic matter leached from the sediments. Copper toxicity, on a water concentration basis, was also lower in the spiked sediment, as compared to the water-only toxicity test. In this case, however, the overlying water concentration of Cu in the un-spiked sediment was still lower than the 4 week LC_{25} in water-only tests. The water concentration data in Tables 3 and 4, therefore, accurately rule out the likelihood of Cu toxicity in Manitouwadge and Hamilton Harbour sediments. However, Zn toxicity is overestimated in sediment tests conducted under these conditions if it is inferred from concentrations of total Zn causing toxicity in water-only exposures.

It is likely that the accuracy of prediction of toxicity from water concentrations could be greatly improved by using regression relationships between waterborne metal toxicity and chemical characteristics of the overlying water. For example, Welsh et al. (1996) were able to predict acute Cu toxicity to fathead minnows in a range of soft-water lakes from a regression of the LC_{50} against pH, dissolved organic matter and calcium. It is likely that such a model, if developed for *Hyalella*, would explain much of the difference in the LC_{25}s for Zn and Cu between water-only and spiked-sediment exposures (Table 4). Toxicity in sediment tests with this species could then be predicted more reliably from the overlying water concentrations. Alternatively, increasing the water to sediment ratio in the toxicity test can, at least in some cases, reduce the effect that organic matter or other chemicals leaching from the sediment have on the concentration of metal in water associated with toxicity. For example, the LC_{50} for Pb based on overlying water in Pb-spiked sediment toxicity tests was much lower in test chambers with a 67:1 water to sediment ratio (43 $nmol\ l^{-1}$) than with a 4:1 ratio (126 $nmol\ l^{-1}$), in spite of the fact that toxicity was identical on a sediment concentration basis (35 $\mu mol\ g^{-1}$) and toxicity was due to dissolved Pb alone (i.e. toxicity to animals caged above the sediments was equal to that of sediment exposed animals). At the low water to sediment ratio, the dissolved organic carbon concentration was more than 2-fold higher than in the high water to sediment treatment, probably resulting in a much higher Pb-complexing capacity, and hence a higher LC_{50} (Borgmann and Norwood, 1999).

Considerable success has recently been obtained in predicting the acute lethality of metals to fish by modeling the uptake of metals on fish gills (e.g. Playle et al., 1993). This approach is similar to the free ion activity modeling approach, but it explicitly examines the interaction between metal ions in water, the receptor site on the organism for metal uptake, and interactions with other ions and complexing agents in the water. This represents a more mechanistically based modeling approach than that described above, and has been considered as one of the most promising approaches for replacing the Water Quality Criteria now in use (Wood et al., 1997). By extension to chronic toxicity, and to invertebrates, it is possible that such an approach could also be used to predict

Table 4
Zinc and copper concentrations as $\mu mol\ l^{-1}$ in overlying water for un-spiked Hamilton Harbour sediments and 4-week lethal water concentrations resulting in 25% mortality of *Hyalella azteca* in water-only and in metal-spiked sediment toxicity tests (LC_{25}). Data from Borgmann and Norwood (1997a)

	Zn	Cu
Un-spiked sediment	1.83	0.24
4-week LC_{25}, water-only exposure	1.70	0.33
4-week LC_{25}, spiked sediment exposure	3.29	1.36

metal-induced toxicity from water to benthic organisms (Wood et al., 1997). This methodology is similar to the use of bio-accumulation–toxicity relationships discussed earlier, in that toxicity is related directly to metal accumulation, but the criteria for predicting effects are based on water concentrations and the appropriate model for accumulation, rather than on the body concentration directly.

The measurement of concentrations in water is the preferred method for assessing the contribution of some chemicals, such as ammonia, to sediment toxicity. It is generally not feasible to measure body concentrations of ammonia and relate these to sediment toxicity. This could be possible if free ammonia in the blood could be measured, but this would require sophisticated equipment and analysis of blood from live animals, a difficult prospect with small invertebrates. Unlike metals, aqueous concentrations of ammonia are not simply a function of equilibrium partitioning between water and sediment, but are controlled by the rate of breakdown of nitrogenous organic matter by bacteria. Fortunately, ammonia toxicity to *Hyalella* can readily be estimated from ammonia, Na, K and pH in the water (Borgmann and Borgmann, 1997).

The use of overlying water for predicting toxicity in sediment toxicity tests is somewhat similar to the concept of extracting sediments with various reagents to measure weakly bound and potentially bio-available fractions of metals. For example, metal accumulation by freshwater mussels is a function of one or more of the relatively easily extracted fractions, rather than total metal (Tessier et al., 1984). The metal concentrations in the overlying water at the end of the toxicity test represent the most weakly bound (i.e. extractable with the bioassay water) and presumably most bio-available fraction of the metals. Metal concentrations in the overlying water during static toxicity tests are generally controlled by equilibration with metal in the sediment (e.g. Borgmann and Norwood, 1999). Consequently, the presence of the animals themselves would not usually be expected to affect the metal concentration in the water because metal removed from the water during bio-accumulation would be replaced by re-equilibration between the water and the sediment. The advantage of measuring water concentrations in sediment toxicity tests is that the chemical 'extraction' is performed at the same time as the toxicity test, thereby ensuring that the chemical measurements are directly comparable to toxicity measurements in each test.

3.2. Identification of toxic metals using sediment spiking

Another approach for identifying potentially toxic metals in sediments, which should be relatively robust, involves spiking the sediments with additional metal and repeating the toxicity test. A convenient amount of metal for spiking is roughly the amount of metal already present. A single metal spike (i.e. one test concentration) would be sufficient. The first step in such an approach would be to measure the concentrations of metals in the sediment sample. Each experimental treatment would then include a spike of one metal resulting roughly in a doubling of the concentration of that metal. If no change in toxicity occurs relative to the un-spiked sample, then it can reasonably be assumed that the metal which was spiked is not causing toxicity. If an increase in toxicity occurs, then the metal added in the spike is either responsible for toxicity in the un-spiked sample, or is probably present at concentrations almost high enough to cause toxicity. An example is shown in Table 5. When Hamilton Harbour sediments were spiked with sufficient metal to increase Zn from 23 to 41 μmol g^{-1}, or Cu from 1.2 to 4.7 μmol g^{-1} (metals were spiked separately, not simultaneously), no increase in mortality was observed. Neither of these metals is, therefore, likely to contribute to chronic toxicity in this sediment sample. The actual LC_{25}s for spiked sediments in this study were 54 μmol Zn g^{-1} and 16 μmol Cu g^{-1} (Borgmann and Norwood, 1997a). If the Zn concentration had been higher (e.g. 41 μmol g^{-1} in un-spiked sediment), spiking with additional Zn would have caused increased mortality (e.g. 33% survival at

Table 5
Toxicity of un-spiked Hamilton Harbour sediments and sediments spiked singly with either Cu or Zn to *Hyalella azteca* in 4-week chronic exposures and metal concentration. Data from Borgmann and Norwood (1997a)

	Zn	Cu
Metal in un-spiked sediment (μmol g^{-1})	23	1.2
Metal in spiked sediment (μmol g^{-1})	41	4.7
Survival in un-spiked sediment (%)	91	91
Survival in spiked sediment (%)	94	95

70 μmol Zn g^{-1}, Borgmann and Norwood, 1997a). In this case, the Zn concentration would have been very close to the toxic threshold and the spiked toxicity test would have been positive, even though the metal was not actually toxic in the un-spiked sediment.

Sediment spiking does not need to be conducted for every metal in a sample. It is most likely to be necessary for the essential metals Cu and Zn, which are always present in relatively high concentrations in animal tissues and for which there may be a negligible or difficult-to-quantify increase in body concentration associated with the onset of chronic toxicity. For non-essential metals the background tissue concentration is often well below the toxic threshold, making it relatively easy to rule out their contribution to toxicity using the body-concentration approach.

A disadvantage of the sediment spiking approach is that the bio-availability of the metal added in the spike cannot be assumed to equal the bio-availability of the metal already present. Some of the metal in the un-spiked sample might be in a very tightly bound form which is much less available than the ionic form added. A doubling of total metal concentration in the sediment by spiking could, therefore, result in much more than a doubling in bio-available metal. Also, it will not be possible to distinguish between metals which are actually causing toxicity, or metals which, though close to the toxic threshold, are non-toxic. For example, if toxicity is already present in a sample, but is not due to a metal which is just below the toxic threshold, then spiking with that metal may increase the metal concentration to above the toxic threshold and cause increased mortality. It would be incorrect to interpret the original toxicity to be due to that metal. Consequently the lack of an increase in toxicity following spiking can be used as evidence that the metal used for spiking is not the cause of toxicity in the original sample, but the reverse is not necessarily true.

4. Critical issues in assessing metal impacts

4.1. Role of body concentrations, water concentrations, and sediment spiking in a full assessment of metal impacts

The three approaches described above (body concentrations, water concentrations and sediment spiking) are all designed to quantify the potential bio-availability and biological impact of metals in sediments. All are extensions of standard laboratory sediment toxicity tests designed to identify cause and effect relationships. Each of the these approaches has its own advantages and disadvantages. For comparison, some of these are summarized in Table 6. The first approach, the body-concentration approach, can sometimes also be applied directly to wild animals if representatives of the test species are found at the sediment collection site (e.g. Borgmann and Norwood, 1997b). However, such measurements should probably be supplemented with tests with standard laboratory animals, at least until the bio-accumulation response of laboratory and wild animals can be compared.

The methods listed in Table 6 can be used effectively as part of a full assessment of the environmental impact of metals. There are four critical questions which must be asked when determining if the production or use of metals is affecting aquatic ecosystems. These have been listed as guiding questions under Canada's Aquatic Effects Technology Evaluation program (AETE, 1997), but are applicable to most cases of environmental contamination: 1. Are contaminants getting into the system? 2. Are contaminants bio-available? 3. Is there a measurable response? 4. Are the contaminants causing this response?

These questions can be answered for metals by, respectively:

1. Measuring metal concentrations in water and sediments, including the examination of geographic distribution and sediment profiles for historical trends.
2. Measuring metals bio-accumulated by animals directly, or metals in the bio-available fraction if this can be identified. Measurement of metals in the overlying water in the field or during toxicity tests with sediments is likely to be a better measure of bio-available metal than total metal in the sediment (e.g. the water-concentration approach, Table 6).
3. Determining changes in benthic community structure and/or measuring sediment toxicity using standard chronic toxicity test procedures.
4. Comparing bio-accumulation (item 2 above) with bio-accumulation–toxicity relationships determined

Table 6
Advantages and disadvantages of three different approaches to assessing the importance of metals in sediments

Advantages	Disadvantages
Use of bio-accumulation–toxicity relationship (the body-concentration approach)	
I. Variations in metal bio-availability in water or sediment are automatically taken into account. Where applicable, this is the most direct and simple approach to identifying metals responsible for toxicity	I. This approach requires data on the bio-accumulation–toxicity relationship for each metal as obtained from, for example, metal-spiked sediment toxicity tests. However, these data need to be collected only once, after which they can be applied to multiple sites
II. The potential toxicity of a large number of metals can be examined simultaneously once background data on the toxicity–bio-accumulation relationship are available for each metal. Tissue samples from animals exposed during toxicity tests can also be dried and stored indefinitely for future analysis of additional metals in case some potentially toxic metals have been overlooked, or if time series trends are of interest	II. This approach will not work for metals which are regulated, or for animal species which sequester metals in large amounts in non-toxic forms within the body, because this precludes the development of a clear toxicity–bio-accumulation relationship. Background data on the physiology of metal uptake and retention by the species of interest are required
	III. The standard toxicity test may need to be extended to include a period of time in clean sediments or water to allow for gut clearance before measurement of body concentrations. An estimate of gut clearance times is required for the test organism. It might also be necessary to repeat the toxicity test using older animals and a shorter exposure period for sediments which result in 100% mortality during the full chronic test in order to provide surviving animals for body concentration analyses
Use of dissolved metal concentrations in toxicity-test water (the water-concentration approach)	
I. Substantial information on the possible identity of toxic metals can be obtained simply by measuring metal concentrations in the overlying water during sediment toxicity tests. This requires relatively little additional effort	I. The variation in metal bio-availability caused by changes in the overlying water is not taken into account. The method is, therefore, less direct than the body-concentration approach, and can, for example, result in overestimation of metal toxicity if organic matter leaching from the sediments reduces metal toxicity
II. This approach will work even for metals which are regulated or sequestered in non-toxic form in animal tissues because it is independent of the toxicity–bio-accumulation relationship	II. Possible toxicity caused by direct exposure to the solid phase is ignored. This is especially important for species which may obtain a substantial body concentration of metals from ingested sediment
Use of sediment spiking (the sediment-spiking approach)	
I. The technique is very simple in concept and should be robust	I. Extra toxicity tests must be conducted with spiked sediments. However, only one spike concentration needs to be tested for each metal, unless more detailed information is desired (e.g. to determine what the LC_{50} would be for a metal in that sample)
II. No background data are required on tissue or water concentrations responsible for causing metal toxicity in the test species	II. It is not possible to distinguish between metals which are actually causing toxicity, and metals which are close to the toxic threshold but non-toxic. The sediment-spiking approach is, therefore, more definitive in ruling out, rather than proving, metal-induced toxicity
III. Variations in metal bio-availability between sediment samples are automatically taken into account because each sediment sample is spiked separately rather than being compared to a reference sediment or a sediment quality guideline	III. The biological impact of the metal already present could be overestimated if the bio-availability of the metal added in the spike is greater than that of the metal already present

in separate experiments with spiked water or spiked sediment toxicity tests (the body-concentration approach), or using the water-concentration or sediment-spiking approach (Table 6).

The combination of numbers 1 and 3 (chemical measurements + community structure analysis + sediment toxicity testing) is what is frequently done during the Sediment Quality Triad approach (Chapman, 1990). This is far superior to relying on sediment chemistry alone (e.g. comparison to sediment quality criteria), but still addresses only 2 of the 4 critical questions. A full assessment of the biological impact of metals requires a link between observed effects and measured concentrations, and this requires methods such as those summarized in Table 6.

4.2. Relationship to sediment quality criteria

In order to assess the potential impact of contaminated sediments on aquatic biota, attempts have been made to establish sediment quality guidelines, objectives or criteria based on chemical concentrations alone. For example, Environment Canada has derived sediment quality assessment values such as the threshold effect level (TEL) and the probable effect level (PEL), concentrations at which biological effects are rarely (<TEL), occasionally (>TEL, <PEL), or frequently (>PEL) observed (Smith et al., 1996). These guidelines were derived by comparing sediment chemical analyses with biological effects (impaired natural communities or sediment toxicity), and the TEL is recommended as an interim sediment quality guideline. The province of Ontario has made use of similar guidelines (Persaud et al., 1993).

It is extremely important to remember that these assessment values are based on correlations and cannot be used to infer cause and effect relationships (Smith et al., 1996). For example, if the PEL for Cd is exceeded and sediment toxicity is observed, this does not mean that Cd is the toxic agent. It is quite possible that Cd, on average, correlates with another metal or other toxic agent in sediments impacted by human activity. The PEL for Cd means that, for the sediments for which this criterion was derived, toxicity was frequently observed if the PEL was exceeded, regardless of the identity of toxic agent. Such sediment quality criteria cannot, therefore, be used to assess the biological significance of individual metals in a given sediment sample. Such an assessment can only be made using methods such as those in Table 6.

Another feature of sediment quality criteria based on total metals in sediments is that they do not take variations in metal bio-availability into account. Consequently, even for those metals which are actually responsible for toxicity at concentrations near the guideline criterion (this might be elucidated in future research), the criterion can only be used as a rough guide to possible effects. The methods listed in Table 6, on the other hand, are deliberately designed to take variations in metal bio-availability into account, resulting in more accurate assessment of the biological impact of specific metals at specific sites.

4.3. Relationship to toxicity identification and evaluation procedures (TIEs)

The approaches outlined here can be used as part of, but are not limited to, toxicity identification and evaluation procedures (TIE) such as those reviewed by Ankley and Schubauer-Berigan (1995). In a TIE, toxicity could be due to any chemical, not just metals. The methods listed in Table 6 can be part of the TIE if metals have been identified as a possible cause of toxicity (e.g., if addition of EDTA reduces toxicity). However, a traditional TIE is normally done only at sites where toxicity has been identified. The approaches listed in Table 6 can also be used in the absence of toxicity to quantify the relative bio-availability of metals at non-toxic sites and to identify sites which may warrant further monitoring because metal bio-availability is close to a toxic threshold. This allows differentiation between sites which are potentially at risk and sites where metal concentrations are well below the toxic threshold. The methods in Table 6 can, therefore, be used to provide early warning of potential problems before actual toxicity occurs.

5. Summary

Because large variations are observed in metal bio-availability in water or sediment, accurate prediction of metal-induced toxic effects from total metal concentrations obtained in the field are not possible. Traditional environmental assessment techniques, such as observations of changes in aquatic communities in the field,

toxicity tests of various kinds, and measurement of total metal concentrations in water or sediment, can demonstrate environmental contamination and effects, but cannot be relied upon to identify accurately cause and effect relationships. One of the most powerful methods of identifying such cause and effect relationships is the measurement of metal concentrations in toxicity test organisms with comparison to bio-accumulation–toxicity relationships determined in metal-spiking experiments. Alternative strategies include measurement of dissolved metal in overlying water and spiking of test sediments with metals. The advantages and disadvantages of these three approaches are summarized in Table 6.

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