

**LITERATURE REVIEW REPORT:  
POSSIBLE MEANS OF  
EVALUATING THE BIOLOGICAL  
EFFECTS OF SUB-AQUEOUS  
DISPOSAL OF MINE TAILINGS**

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Literature Review Report:  
Possible Means of Evaluating the Biological Effects  
of Sub-Aqueous Disposal of Mine Tailings

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## EXECUTIVE SUMMARY

This review identifies promising geochemical and ecotoxicological approaches that might be used to monitor the biological effects of the sub-aqueous disposal of reactive mine tailings.

Submerged mine tailings, and their constituent metals, may affect aquatic life in two ways: indirectly (i.e., by leaching of the metals into the ambient water, followed by their assimilation from the aqueous phase), and directly (e.g., in macrofauna, by ingestion of the tailings and assimilation of the metals from the gut). Both routes of metal exposure are considered. The metals that have been considered are those that are commonly present in reactive mine tailings, that are recognized as potentially toxic at low concentrations to aquatic biota, and that exist in natural waters as dissolved cations (e.g., Cd, Cu, Ni, Pb, Zn).

### *Geochemical considerations*

- To evaluate indirect exposure, one needs to estimate metal concentrations in sediment pore waters (i.e.,  $[M]_j$ ). Such concentrations are thought to reflect the metal's chemical potential at the sediment-water interface. Changes in this chemical potential will affect the metal's bioavailability.
- Two approaches can be used to estimate  $[M]_j$ : one applies to oxic conditions and assigns control of  $[M]_j$  to sorption reactions on such sorbants as Fe-, Mn - oxyhydroxides or sediment organic matter; the second applies to anoxic conditions and assumes that  $[M]_j$  is controlled by precipitation-dissolution reactions with reactive amorphous sulfides (Acid-Volatile-Sulfides, AVS).
- The two approaches differ in their choice of reactions controlling metal solubility in sediment pore waters. This divergence stems from different concepts of what constitutes biologically important sediments - fully oxidized, surficial sediments (where amorphous sulphide levels should be vanishingly low and  $[M]_j$  should be controlled by sorption reactions) vs. partially oxidized, sub-oxic sediments (where significant AVS levels would be expected to persist, and exchange reactions with amorphous sulphides would control metal partitioning between dissolved and solid phases).
- Choosing between the two approaches is not straightforward, even for natural sediments. In the real world, due to small scale spatial heterogeneity, distinction between oxic and anoxic sediments is often blurred. Most aerobic benthic organisms survive in sediments that are underlain or even surrounded by anaerobic material, which constitute a potential source of AVS.
- Depending on which geochemical approach proves more appropriate for deposited mine tailings (i.e., fully or partially oxidized conditions at the tailings-water interface), it should be possible to predict  $[M]_j$  based on the geochemistry of the tailings after diagenesis and admixture of natural particulate material.

*Interactions between dissolved trace metals and aquatic organisms*

- Qualitative evidence suggests that the total aqueous concentration of a metal is not a good predictor of its bioavailability. The metal's speciation greatly affects its availability to aquatic organisms.
- A convincing body of evidence supports the tenet that the biological response elicited by a dissolved metal is usually a function of the free metal ion concentration,  $M^{Z+}$ .
- This Free-Ion Activity Model (FIAM) should apply to aquatic organisms that do not assimilate particulate material (e.g., rooted aquatic plants), and to organisms that do assimilate particulate material but for which the dissolved phase remains the primary vector for metal uptake.
- However, most experiments designed to test the FIAM have been performed in the laboratory at fixed pH, with divalent metals (Cu, Cd, Ni, Pb, Zn), in artificial (inorganic) media or in filtered sea water, and in the presence of known quantities of synthetic ligands. The applicability of the FIAM in natural waters, in the presence of natural dissolved organic matter (DOM), is poorly documented.
- Two types of study have been carried out to test the applicability of the FIAM in the presence of sediments: (1) laboratory bioassays on spiked sediments or on natural sediments collected from known contaminated sites; and (2) field surveys of indigenous benthic organisms. Both approaches support the general idea that benthic organisms respond to the free-metal ion concentration at the sediment-water interface.

*Interactions between particulate trace metals and aquatic organisms*

- Benthic organisms that ingest particles tend to select the smaller and lighter particles in their environment. This nutritional strategy results in the ingestion of particles that tend to be enriched in metals.
- Assimilation of particle-bound metals will normally involve their conversion from particulate to dissolved form in the gut, followed by their facilitated diffusion across the intestinal membrane. Digestive processes and chemical conditions prevailing within the intestinal tract thus assume considerable importance (e.g., pH, digestion times, redox status).
- Based on feeding experiments where metal uptake from different sediment phases was monitored, the efficiency of uptake of a given metal varied greatly among different model sediments, and relative availability from a given sink varied from metal to metal. Thus, one cannot generalize that metals are more available from phase A (e.g., organic detritus) than from phase Z (e.g., Mn(IV) oxyhydroxide) - while the sequence  $A > Z$  may be true for one metal, it will not necessarily hold for the next.
- Differences in metal availability tend to be inversely related to the strength of metal binding to particulates. Sediments that exhibited the highest affinity for a metal (i.e., that released the least amount of metal back into solution) were also the substrates from which metal bioavailability was the least.

- From these studies it is clear that the physicochemical form of a sediment-bound metal affects its availability in the digestive tract of the marine deposit feeder *Macoma balthica*. However, it is premature to attempt to generalize these results to all deposit-feeders.
- The results of the feeding experiments with *M. balthica* are admittedly qualitative. Nevertheless, there is an interesting parallel between the original "affinity" concept (according to which the availability of a particle-bound metal is inversely related to the strength of the metal-particle association) and more recent suggestions that the concentration of a metal in the interstitial water can be taken as a measure of its chemical potential in surficial sediments and thus its availability. This inference seems intuitively reasonable, provided that the chemical environment within the animal's digestive tract is similar to that in its immediate environment ( i.e., provided the chemical potential of a metal doesn't change drastically on passing from outside environment into the digestive system). In this context, better knowledge of the chemistry of invertebrate digestion will be very useful.

#### *Biochemical indicators of metal-induced stress*

- Traditionally, attempts to define the impacts of contaminants on aquatic ecosystems have involved laboratory experiments under defined conditions (toxicity tests) and, to a lesser extent, field observations on impacted indigenous populations. An alternative and complementary approach involves the use of biochemical indicators to monitor the response of individual organisms to toxic chemicals and to provide a measure of ecosystem health.
- For metals, much of the attention in the area of biochemical indicators has focused on metal-binding proteins, in particular on metallothionein (MT) and metallothionein-like compounds.
- Given its molecular properties, and present knowledge of its role in metal uptake, transport, storage and excretion, metallothionein offers considerable potential as a contaminant-specific biochemical indicator of metal exposure and/or stress.
- Possible approaches include: (i) measurement of metallothionein concentrations as an indicator of prior exposure to toxic metals, and (ii) examination of the relative distributions of toxic metals in cytosolic ligand pools to evaluate metal stress at the biochemical level.
- Field evidence in support of the first approach is slowly accumulating in the eco-toxicological literature. In studies involving the sampling of indigenous populations of aquatic animals from sites chosen to represent a spatial (metal) contamination gradient, the concentrations of metallothionein-like proteins were consistently higher at the more contaminated sites.
- Regarding the second possible use of MT, i.e. to evaluate metal stress at the biochemical level, responses of aquatic organisms to excess metal have proven more diverse than originally postulated. Thus, toxic metal distributions in the cytosolic fraction cannot yet be used to evaluate metal stress at the biochemical level.

*Effects of metals at the population and community levels*

- Structural components of ecosystems are often considered the most sensitive indicator of disturbance. These components include communities (the biotic components of an ecosystem) and populations (the members of a species that occupy the same habitat and can possibly interbreed).
- Recent studies also suggest that chronic exposure to low levels of contaminants often results in severe effects on populations and communities in aquatic environments. Thus, community and population data are essential for the prediction and monitoring of these effects.
- However, the effects of natural population cycles, climate and other environmental factors not related to contamination must be accounted for if the impacts of contaminants on populations and communities are to be properly assessed. This can be accomplished by the use of representative reference sites and collection of sufficient background chemical and biological data.
- Certain **community indices** are more useful than others in detecting the impacts of contaminants such as metals or acids.
  - (i) Changes in the algal community (number of species, diversity, species composition, dominant species) are often observed. However, changes in dominance may also be due to other factors, such as the availability of nutrients, reduced grazing pressure, or the development metal tolerance by normally sensitive taxa. The most useful measures appear to be species composition and richness.
  - (ii) Few studies have examined impacts of metals on lake communities of crustacean zooplankton, despite their importance as a prey item. Decreases in diversity and in total biomass, as well as changes in dominance among crustacean zooplankton communities have been observed.
  - (iii) The benthic community is in intimate contact with both water and sediment phases and has been extensively used as an indicator of metal pollution in streams. In some studies of heavily impacted lakes, the density of benthic organisms proved to be a useful indicator, though this may not be the case in less severely contaminated habitats. Measurements of diversity have proven of limited usefulness for the detection of community changes. Proportions of tolerant and sensitive taxa have also been used as gradational indices of metal stress. However, the classification of entire taxa as tolerant or sensitive may ignore important differences which exist within them. Improved methods for the taxonomic differentiation of benthic animals are needed both to improve the relationship of community composition with the degree of metal stress and to place these effects in an ecological context.
  - (iv) Most studies involving fish communities have involved listing the species present (community composition), measuring the species richness (the number of species) or using associations of species, which are considered to prefer certain habitats, as indicative of water quality.



- Fish **populations** are not necessarily the most sensitive predictors of the future state of a system. However, there are certain advantages to their use, notably the relative facility of collecting the data necessary for such studies (age of individuals, reproductive status, growth, condition) and the existence of historical records for comparison. Furthermore, population data are essential to relate chronic toxicity, often observed in individuals in the field, to effects at the level of the population. Different strategies (*r* vs. *k*) may be observed in populations adapting to stress.
- Many natural fish populations appear to be tolerant of waterborne metal concentrations that are acutely toxic in laboratory studies. This apparent discrepancy may be related to differences in metal speciation. Such explanations remain speculative, however, since in most field studies the chemistry of the exposure medium (sediments, water column) was not sufficiently defined.

## SOMMAIRE

Cette revue identifie des approches géochimiques et écotoxicologiques prometteuses qui pourraient être utilisées pour surveiller les effets biologiques de la disposition subaquatique des résidus miniers réactifs.

Les résidus miniers submergés, ainsi que leurs constituants métalliques, peuvent influencer sur la vie aquatique de deux façons: indirectement (p. ex. par la lixiviation de métaux dans l'eau ambiante, suivie par leur assimilation à partir de la phase aqueuse) et directement (p. ex. dans la microfaune, par ingestion des résidus et l'assimilation des métaux à partir des voies intestinales). Ces deux voies d'exposition aux métaux sont examinées. Les métaux qui ont été retenus sont ceux qu'on retrouve communément dans des résidus miniers réactifs et qui sont connus comme étant potentiellement toxiques aux faibles concentrations pour le biote aquatique, et qui existent dans les eaux naturelles à l'état de cations dissous (p. ex. Cd, Cu, Ni, Pb, Zn).

### *Considérations géochimiques*

- Pour évaluer l'exposition indirecte, il faut estimer les concentrations des métaux dans les eaux interstitielles des sédiments (c.-à-d.  $[M]_i$ ). On croit que de telles concentrations reflètent le potentiel chimique du métal à l'interface sédiments-eau. Les changements de ce potentiel chimique influenceront sur la biodisponibilité du métal.
- Deux approches peuvent être utilisées pour évaluer  $[M]_i$ : l'une s'applique aux conditions oxiques et attribue la régulation de  $[M]_i$  à des réactions de sorption sur des sorbants comme des oxyhydroxydes de Fe, ou de Mn, ou des matières organiques sédimentaires, alors que la deuxième s'applique à des conditions anoxiques et suppose que les  $[M]_i$  sont déterminées par des réactions de précipitation-dissolution avec des sulfures amorphes réactifs (Sulfures-Volatils en Milieu Acide, SVMA).
- Ces deux approches diffèrent par leur choix des réactions déterminant la solubilité des métaux dans les eaux interstitielles des sédiments. Cette divergence provient de différences théoriques en ce qui trait à la nature des sédiments biologiquement importants, qui sont soit des sédiments superficiels et complètement oxydés (dans lesquels les teneurs en sulfure amorphe devraient être très faibles et où les  $[M]_i$  devrait être déterminées par des réactions de sorption), soit des sédiments suboxiques partiellement oxydés (où des teneurs significatives en SVMA devraient persister, et où des réactions d'échange avec des sulfures amorphes devraient déterminer la séparation des métaux entre les phases dissoutes et solides).
- Le choix entre ces deux approches n'est pas simple, même pour les sédiments naturels. En conditions réelles, à cause de l'hétérogénéité spatiale à petite échelle, la distinction entre les sédiments oxiques et anoxiques est souvent peu évidente. La plupart des organismes benthiques aérobies survivent dans les sédiments recouverts ou même entourés de matières anaérobies, qui constituent une source possible de SVMA.
- Selon que l'une ou l'autre de ces approches géochimiques est plus appropriée pour les résidus miniers (c.-à-d. aux conditions d'oxydation entières ou partielles à l'interface résidus-eau), il devrait

être possible de prévoir les valeurs de  $[M]$ , en se basant sur la géochimie des résidus après diagenèse et mélange des matières particulaires naturelles.

*Interactions entre les métaux à l'état de traces dissous et les organismes aquatiques*

- Les résultats obtenus avec des données quantitatives suggèrent que la concentration aqueuse totale d'un métal ne permet pas une bonne prévision de sa biodisponibilité. La différenciation des espèces d'un métal influe fortement sur sa disponibilité pour les organismes aquatiques.
- Un ensemble impressionnant de résultats appuie l'hypothèse selon laquelle la réponse biologique causée par un métal dissous est habituellement en fonction de la concentration de l'ion métallique libre,  $M^{Z+}$ .
- Ce Modèle de l'activité des ions libres (MAIL) devrait s'appliquer aux organismes aquatiques qui n'assimilent pas de matières particulaires (p. ex. les plantes aquatiques à racines), ainsi qu'aux organismes qui assimilent les matières particulaires mais pour lesquels la phase dissoute reste le principal vecteur pour l'absorption des métaux.
- Toutefois, la plupart des expériences conçues pour tester le MAIL ont été effectuées en laboratoire à des pH fixes, avec des métaux divalents (Cu, Cd, Ni, Pb, Zn) dans des milieux artificiels (inorganiques) ou dans l'eau de mer filtrée, et en présence de quantités connues de ligands synthétiques. L'applicabilité du MAIL aux eaux naturelles, en présence de matières organiques dissoutes (MOD), est mal documentée.
- Deux types d'études ont été effectuées pour vérifier l'applicabilité du MAIL en présence de sédiments: 1) des bioessais en laboratoire effectués avec des échantillons enrichis et des sédiments naturels prélevés à des endroits contaminés connus et 2) des relevés sur le terrain d'organismes benthiques indigènes. Ces deux approches appuient la théorie générale selon laquelle les organismes benthiques répondent aux concentrations d'ions métalliques libres à l'interface sédiments-eau.

*Interactions entre les métaux à l'état de traces sous forme de matières particulaires et des organismes aquatiques*

- Les organismes benthiques qui ingèrent des particules tendent à sélectionner les particules les plus petites et les plus légères de leur milieu. Cette stratégie nutritionnelle entraîne l'ingestion de particules qui tendent à être enrichies en métaux.
- L'assimilation de métaux liés à des particules est normalement à l'origine de leur conversion de la forme particulaire à la forme dissoute dans les voies intestinales, suivie par leur diffusion facilitée à travers la membrane intestinale. Les processus digestifs et les conditions chimiques prévalant dans les voies intestinales ont donc une importance considérable (p. ex. pH, période de digestion, état d'oxydo-réduction).
- En se basant sur des expériences d'alimentation pendant lesquelles l'absorption de métaux de différentes phases sédimentaires était surveillée, on a conclu que l'efficacité de l'absorption d'un

métal donné variait fortement d'un modèle de sédiments à l' autre, et la disponibilité relative d' un puits donné variait d'un métal à l' autre. Par conséquent, il n' est pas possible de tirer des conclusions générales disant par exemple que les métaux de la phase A sont plus disponibles (p. ex., détritiques organiques) que ceux de la phase Z (p. ex. oxyhydroxyde de Mn(IV)) - alors que la séquence A > Z peut être vraie pour un métal, mais ne l'est pas nécessairement pour un autre.

- Les différences de disponibilité d'un métal tendent à varier en rapport inverse avec la force du lien entre ce métal et les matières particulaires. Les sédiments qui présentaient la plus forte affinité pour un métal (c.-à-d. qui libéraient le moins de métal en solution) étaient également les substrats à partir desquels la biodisponibilité de ce métal était la plus faible.
- À partir de ces études, il est clair que la forme physico-chimique d'un métal lié à un sédiment influe sur sa disponibilité dans les voies intestinales de l'organisme limnivore *Macoma balthica*. Toutefois, il serait prématuré de tenter d'extrapoler ces résultats à tous les limnivores.
- Les résultats des expériences d'alimentation effectuées avec *M. balthica* sont, de l'aveu des auteurs, qualitatifs. Néanmoins, il existe un parallèle intéressant entre le concept original d' "affinité" (selon lequel la disponibilité d'un métal lié à une particule est en rapport inverse avec la force du lien métal-particule) et des indications plus récentes selon lesquelles la concentration d'un métal dans l'eau interstitielle peut être utilisée comme une mesure de son potentiel chimique dans les sédiments de surface et par conséquent, comme une mesure de sa disponibilité. Cette conclusion semble vraisemblable, à condition que l'environnement chimique à l'intérieur des voies digestives d'un animal soit semblable à celui de son environnement immédiat (c.-à-d., à condition que le potentiel chimique d'un métal ne change pas de façon importante quand celui-ci passe d'un environnement extérieur au système digestif). Dans ce contexte, de meilleures connaissances de la chimie de la digestion des invertébrés seraient très utiles.

#### *Indicateurs biochimiques du stress causé par un métal*

- Ordinairement, les tentatives visant à déterminer les répercussions des contaminants, sur les écosystèmes aquatiques sont basées sur des expériences en laboratoire dans des conditions définies (essais de toxicité) et, dans une moindre mesure, sur des observations sur le terrain portant sur des populations indigènes ayant subi des répercussions. Une approche complémentaire et différente est basée sur l'utilisation d'indicateurs biochimiques pour surveiller la réaction d'organismes particuliers aux produits chimiques toxiques et pour donner une mesure de la santé de l'écosystème.
- Dans le cas des métaux, une bonne partie des travaux dans le domaine des indicateurs biochimiques ont porté sur des protéines liant des métaux, et plus particulièrement sur la métallothionéine (MT), et sur les composés de type métallothionéine.
- Étant donné ses propriétés moléculaires, ainsi que les connaissances actuelles sur son rôle dans l'absorption, le transport, le stockage et l'excrétion des métaux, la métallothionéine offre d'excellentes possibilités comme indicateur biochimique spécifique pour un contaminant de l'exposition à un métal et (ou) du stress causé par celui-ci.

- Parmi les approches possibles, notons: i) la mesure des concentrations de métallothionéine comme indicateur de l'exposition antérieure à des métaux toxiques et ii) l'examen des répartitions relatives des métaux toxiques dans les pools de liants cytosoliques afin d'évaluer le stress causé par un métal au niveau biochimique.
- Les données *in situ* à l'appui de la première approche s'accumulent lentement dans la littérature écotoxicologique. Dans les études utilisant l'échantillonnage de populations indigènes d'organismes aquatiques provenant de sites sélectionnés de façon à représenter un gradient spatial de contamination (par un métal), les concentrations de protéines de type métallothionéine étaient régulièrement supérieures dans la plupart des sites les plus contaminés.
- Pour ce qui est de la deuxième utilisation possible de la MT, c.-à-d. l'évaluation du stress causé par un métal au niveau biochimique, les réponses des organismes aquatiques aux excès de métaux ont donné des résultats beaucoup plus diversifiés qu'on ne l'aurait supposé avant les études. Ainsi, les distributions de métaux toxiques dans la fraction cytosolique ne sont pas encore utilisables pour évaluer le stress causé par un métal au niveau biochimique.

#### *Effets des métaux au niveau des populations et des communautés*

- Les éléments structurels des écosystèmes sont souvent considérés comme les indicateurs les plus sensibles des perturbations. Ces éléments comprennent les communautés (les éléments biotiques d'un écosystème) ainsi que les populations (les membres d'une espèce qui occupent le même habitat et peuvent éventuellement se reproduire entre eux).
- Des études récentes suggèrent également que l'exposition chronique à de faibles teneurs en contaminants entraîne souvent des effets importants sur les populations et les communautés dans les environnements aquatiques. Donc, des données sur les communautés et les populations sont essentielles pour la prévision et la surveillance de ces effets.
- Toutefois, les effets des cycles naturels des populations, le climat et d'autres facteurs environnementaux non reliés à la contamination doivent être pris en compte si l'on veut évaluer de façon adéquate les répercussions des contaminants sur les populations et les communautés. Ceci peut être fait par l'utilisation de sites de référence représentatifs ainsi que par la collecte d'une quantité suffisante de données chimiques et biologiques de fond.
- Certains **indices communautaires** sont plus utiles que d'autres pour déceler les répercussions de contaminants comme les métaux ou les acides.
  - i. Des changements dans la communauté algale (nombre des espèces, diversité, composition des espèces, espèces dominantes) sont souvent observés. Toutefois, les changements dans la dominance peuvent également être dus à d'autres facteurs comme la disponibilité des nutriments, une réduction de la pression de broutage ou le développement d'une tolérance pour un métal par un taxon normalement sensible. Les mesures les plus utiles semblent être la composition et la richesse des espèces.

- ii. Peu d'études ont porté sur les répercussions des métaux sur les communautés lacustres de zooplancton crustacéen, en dépit de leur importance alimentaire. On a observé des diminutions de la diversité et de la biomasse totale, ainsi que des changements dans la dominance chez des communautés de zooplancton crustacéen.
  - iii. La communauté benthique est en contact intime avec les phases aqueuses et sédimentaires et a été largement utilisée comme indicateur de la pollution par les métaux dans les cours d'eau. Dans certaines études portant sur des lacs ayant subi de fortes répercussions, la densité des organismes benthiques s'est avérée un utilisateur utile, bien que ce paramètre puisse ne pas être applicable dans des habitats moins contaminés. L'utilité des mesures de diversité s'est avérée d'une utilité limitée pour la détection des changements au niveau des communautés. On a également utilisé les proportions de taxons tolérants et sensibles comme indices pour la gradation du stress causé par les métaux. Toutefois, la classification de taxons entiers en "tolérants" et en "sensibles" peut ignorer des différences importantes qui peuvent exister entre ceux-ci. Des méthodes améliorées de différenciation taxonomique des animaux benthiques sont nécessaires pour améliorer la mesure du rapport entre la composition d'une communauté et le degré de stress causé par les métaux, ainsi que pour replacer ces effets dans un contexte écologique.
  - iv. La plupart des études portant sur les communautés de poisson étaient basées sur la préparation de listes des espèces présentes (composition de la communauté), la mesure de la richesse en espèces (nombre d'espèces) ou l'association d'espèces semblant préférer certains habitats, utilisées comme indicateur de la qualité de l'eau.
- Les **populations** de poisson ne sont pas nécessairement les prédateurs les plus sensibles de l'état futur d'un système. Toutefois, l'utilisation de ces populations présente certains avantages, notamment la facilité relative de la cueillette des données nécessaires pour de telles études (âge des individus, état reproductif, croissance, condition) et l'existence de données historiques permettant des comparaisons. En outre, les données concernant la population sont essentielles pour établir un rapport entre la toxicité chronique, souvent observée *in situ* chez des individus, et des effets au niveau de la population. Différentes stratégies (*r* vs *k*) peuvent être observées chez les populations s'adaptant au stress.
  - Bon nombre de population naturelles de poisson semblent tolérer des concentrations de métaux en milieu aqueux correspondant à des doses toxiques aiguës selon des études en laboratoire. Cet écart apparent peut être dû à des différences de différenciation des espèces de métaux. Toutefois, de telles explications restent hypothétiques, étant donné que dans la plupart des études, la chimie du milieu d'exposition (sédiments, colonne d'eau) n'a pas été suffisamment définie.

## **1. INTRODUCTION**

In 1992 the Rawson Academy of Aquatic Sciences undertook a critical review of the studies conducted to 1991 on the sub-aqueous disposal of mine tailings under the "Mine Environment Neutral Drainage" (or MEND) programme. In its final report, the Scientific Review Team recommended that "selective and additional literature reviews" be undertaken on the biological uptake of metals, their bioaccumulation and their possible toxic effects on aquatic organisms. The present literature review aims to respond to this recommendation.

Given the extensive literature available on metal-organism interactions, and the consequent need to limit the scope of this review, we have focused our attention on the possible biological effects of the sub-aqueous disposal of mine tailings. The basic premise has been that mine tailings, and their constituent metals, may affect aquatic life in two ways: indirectly (i.e., by leaching of the metals into the ambient water, followed by their assimilation from the aqueous phase), and directly (e.g., in macrofauna, by ingestion of the tailings and assimilation of the metals from the gut). Both routes of metal exposure have been considered.

The reasoning that guided our review is summarized in Table 1. Considering the indirect route of exposure (via water), we first consider the reactions that are likely to control metal concentrations,  $[M]_i$ , in the interstitial or pore waters of deposited tailings (**section 2: Geochemical considerations**). Two approaches are described for estimating  $[M]_i$  on the basis of the geochemistry of the tailings after diagenesis and admixture of natural particulate material. Secondly, having considered possible geochemical controls on metal concentrations in pore waters, we address the bioavailability of these dissolved metals (in the pore waters and in the overlying water column, assuming vertical transfer), using as a convenient paradigm the Free-Ion Activity Model (FIAM) of metal-organism interactions (**section 3: Interactions between dissolved metals and aquatic organisms**).

After dealing with dissolved metals (geochemical controls; bioavailability), we focus on those metals that remain in particulate form, i.e. the direct route of exposure (**section 4: Interactions between particulate metals and aquatic organisms**). This reasoning is designed to apply to organisms for which particulate material constitutes an important vector for metal uptake. We explore possible links between digestive processes in benthic organisms and the "bioavailability" of ingested sediment-bound metals, and then review the results of laboratory experiments designed to determine the assimilation efficiencies of metals present in different forms in the "food".

In the final section of the literature review (**section 5: Ecotoxicological considerations**) we adopt a more holistic approach and ask the question "What can the indigenous biota tell us about the availability of the metals in mine tailings?". At the organismal level, we consider the monitoring of metal bioaccumulation *per se*, and the use of biochemical indicators (biomarkers) of metal-induced, sub-lethal stress. We also examine the possibility of monitoring effects at a higher level of organization (population or community).

Table 1: Summary of the reasoning that guided the literature review on the possible biological effects of the subaqueous disposal of reactive mine tailings.

<u>Step</u>	<u>Comments/Rationale</u>
1. Ignore <u>how</u> the tailings came to be deposited, i.e. the disposal operation itself.	In other words, consider only settled tailings.
2. Concentrate on the <u>upper</u> layer of the deposited tailings.	The effective contact zone between the aquatic biota and the deposited tailings (Davis and Luoma 1983; NRCC 1988).
3. In the case of metal exposure via the <u>dissolved phase</u> , consider possible controls on metal concentrations in the interstitial (pore) water, $[M]_i$ .	Two possible approaches: (i) control by sorption reactions on Fe, Mn-oxyhydroxides or on sedimentary organic matter; (ii) control by precipitation as amorphous sulphides.
4. Depending on which approach proves more appropriate for deposited mine tailings, estimate $[M]_i$ based on the geochemistry of the tailings after diagenesis and admixture of natural sediments.	In real world, the distinction between oxic and anoxic sediments is often blurred; most aerobic benthic organisms survive in sediments that are underlain or even surrounded by completely anaerobic sediments.
5. Consider the <u>bioavailability</u> of these dissolved metals (in the pore waters; in the overlying water, assuming vertical transfer), using the Free-Ion Activity Model (FIAM).	FIAM should apply to organisms that do not assimilate particulate material (e.g., rooted aquatic plants), and to organisms that do assimilate particles but for which the dissolved phase remains the primary vector for metal uptake (e.g., molluscs).
6. Having dealt with dissolved metals (geochemical controls; bioavailability), now consider those metals that remain in <u>particulate</u> form. Review results of laboratory feeding experiments designed to determine the assimilation efficiencies of metals present in different forms in the ingested particles.	Applies to organisms for which particles constitute the primary vector for metal uptake. Key factors include the particle size ingested, digestion chemistry in the gut (pH; pE; residence time).
7. To complement the "reductionist" approach outlined in steps 1 to 6, consider a holistic (ecotoxicological) approach.	What can the indigenous biota tell us about the bioavailability of metals in mine tailings? (biochemical indicators of metal exposure; population and community level indicators of metal-induced stress).



Given the very broad subject area to be covered in this report, it is important to define its scope at an early stage. The metals of concern will be those that are commonly present in reactive mine tailings, are recognized as potentially toxic at low concentrations to aquatic biota, and that exist in natural waters as dissolved cations (e.g., Cd, Cu, Ni, Pb, Zn); we will not be dealing with anionic species such as arsenate/arsenite, nor with organometallic species such as methyl-mercury. The ultimate aim of this review is to identify promising geochemical and ecotoxicological approaches that might be used to monitor the biological effects of the sub-aqueous disposal of reactive mine tailings.

## **2. GEOCHEMICAL CONSIDERATIONS**

Given the existence of a sub-aqueous deposit of mine tailings, and ignoring how the tailings came to be deposited (i.e., ignoring the disposal operation itself and considering only the settled, quiescent tailings<sup>1</sup>), one can ask how the metals present in the tailings could become biologically available, how they could affect the aquatic biota. As discussed in detail in an earlier MEND literature review (Rescan 1989), diagenesis /oxidation of the superficial tailings would be anticipated, leading to possible enrichment of the metal concentrations in the interstitial (pore) waters. This reasoning leads naturally to a consideration of the geochemical controls on metal concentrations in pore waters,  $[M]_i$ .

Virtually all the relevant literature in this field has been derived from the study of aquatic sediments. We assume that deposited mine tailings will behave in a similar manner to natural sediments, and that metal concentrations in tailings pore waters are controlled by equilibrium processes. Two approaches can be used to estimate  $[M]_i$  : one applies to oxic conditions and assigns the control of  $[M]_i$  to sorption reactions on such sorbants as Fe-, Mn - oxyhydroxides or sedimentary organic matter; the second applies to anoxic conditions and assumes that  $[M]_i$  is controlled by precipitation-dissolution reactions with reactive amorphous sulfides.

### **2.1 Oxic sediments**

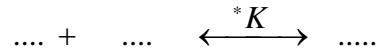
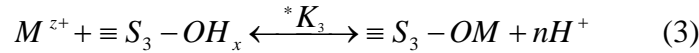
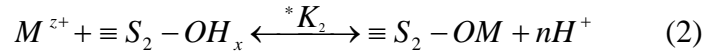
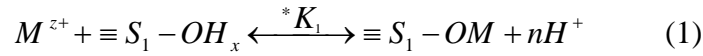
The evidence for sorptive control of dissolved trace metal concentrations under oxic conditions has recently been reviewed by Tessier (1992). With the notable exception of Fe(III) and Mn(IV), measured trace metal concentrations in oxic waters are consistently much lower than those predicted from solubility equilibria involving known solid phases. It follows, therefore, that reactions other than precipitation must be involved in the geochemical control of trace metal concentrations under such conditions. Given the presence in natural sediments of solid phases known to be good sorbants (e.g., Fe oxyhydroxides, Mn oxyhydroxides, sedimentary organic matter), sorption reactions have generally been invoked to explain the observed undersaturation

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<sup>1</sup>As discussed in the earlier review (Rescan 1989), the initial effect of sub-aqueous disposal of mine tailings on the benthic organisms will be largely physical in nature, i.e. a drastic change of habitat that will probably eliminate the benthic community. The rate of recolonization of the submerged tailings will presumably depend on the rate at which organic material (e.g., sedimenting plankton) is incorporated into the (essentially inorganic) tailings, rendering them habitable. The present literature report picks up the story at this point, as it assumes that the tailings have been colonized - in unproductive systems it might take some time to reach this point.

(Schindler 1967). Indeed, in laboratory studies metals added to suspensions of these various solid phases are rapidly removed from solution and the metal concentrations in solution at equilibrium are lower than those predicted from solubility relationships ( $K_{sp}$ ). Similarly, in experiments where natural sediments have been compared with individual sorbants, the dissolved metal concentrations at equilibrium have been shown to respond to changes in various experimental variables (e.g.,  $\Delta pH$ ,  $\Delta[M]$ ,  $\Delta\{\text{sorbant}\}$ ) in much the same manner.

Models for the sorptive control of trace metal concentrations generally assume that the individual sorbants present in the surface sediments compete for the trace metal. The partitioning of the trace metal among the various sorbants can then be described as follows:



$$*K_i = \frac{\{\equiv S_i - OM\} [H^+]^n}{\{\equiv S_i - OH_n\} [M^{z+}]} \quad (4)$$

where S = sorbant (e.g., "S<sub>1</sub>" = Fe(III) oxyhydroxide; "S<sub>2</sub>" = Mn(IV) oxyhydroxide; "S<sub>3</sub>" = sedimentary organic matter);  $\{\equiv S_i - OH_n\}$  = concentration of free binding sites on sorbant "i" ;  $\{S_i - OM\}$  = concentration of sites occupied by metal M;  $[M^{z+}]$  = concentration of the free metal ion;  $*K_i$  = apparent overall equilibrium constant for sorption on substrate "i". Note that here and elsewhere in the text the concentrations of solid phases are indicated by  $\{ \}$  parentheses, whereas concentrations of dissolved species are designated by  $[ \ ]$  brackets; the notation "≡" refers to adsorption sites. Charges on the various solid phases have been omitted for simplicity.

To estimate the sorption constants, we need to be able to determine the variables on the right-hand-side of equation (4) for at least one metal-sorbant combination present in natural sediments. In principle, partial chemical extractions can be used to extract a particular sorbant (e.g., amorphous Fe(III) oxyhydroxides) and its associated metals. With certain simplifying assumptions (described in Appendix 1, and discussed in detail in Tessier (1992)), the ratio  $\{Fe-OM\}/\{Fe-ox\}$  can be substituted in equation (4) for the ratio  $\{\equiv S_i - OM\}/\{\equiv S_i - OH_n\}$ .

$$K_M = \frac{\{Fe - OM\} [H^+]^n}{\{Fe - ox\} [M^{z+}]} \quad (5)$$

Note that  $\{Fe-ox\}$  corresponds to the analytically determined concentration of amorphous iron oxyhydroxides and  $\{Fe-OM\}$  refers to the concentration of metal "M" co-extracted with the Fe-ox sorbant. Values for the remaining variables in equation (5), i.e.  $[H^+]$  and  $[M^{z+}]$ , can be obtained by sampling and analyzing the pore water in equilibrium with oxic surface sediments.

Once again certain simplifying assumption must be made, notably with respect to the speciation of metal "M" in the oxic pore water (i.e., that there is no appreciable complexation of metal "M" by the dissolved organic matter present in the interstitial water - see Appendix 1).

Using field measurements of this type, Tessier (1992) determined conditional constants for the sorption of Cd, Cu, Ni, Pb and Zn on natural amorphous iron oxyhydroxides (Table 2). As anticipated from equations (1) to (3), metal cation sorption is favoured at high pH - the slopes of the log  $K_M$  vs. pH relations shown in Table 2 are all positive.

Table 2: Field-derived equilibrium constants for the sorption of trace metals on amorphous Fe(III) oxyhydroxides (from Tessier 1992).

<u>Metal</u>	<u>Relation</u>
Cd	Log $K_{Cd} = 1.03 \text{ pH} - 2.44$ ( $r^2=0.80$ ; $n=26$ )
Cu	Log $K_{Cu} = 0.64 \text{ pH} + 0.10$ ( $r^2=0.75$ ; $n=39$ )
Ni	Log $K_{Ni} = 1.04 \text{ pH} - 2.29$ ( $r^2=0.87$ ; $n=29$ )
Pb	Log $K_{Pb} = 0.81 \text{ pH} + 0.67$ ( $r^2=0.81$ ; $n=7$ )
Zn	Log $K_{Zn} = 1.21 \text{ pH} - 2.83$ ( $r^2=0.89$ ; $n=41$ )

The proton stoichiometry as calculated from the field measurements (Table 2) is similar to that observed for the surface complexation of metals on synthetic iron oxyhydroxides in laboratory studies. At low pH values the field-derived  $K_M$  values decrease in the sequence  $Pb > Cu > Zn > Ni \approx Cd$  (Tessier 1992); a similar affinity sequence is observed in laboratory sorption studies on synthetic iron oxyhydroxides in well-defined media. This correspondence between field and laboratory measurements can be taken as an indication that sorption phenomena are indeed responsible for controlling dissolved trace metal concentrations under oxic conditions.

Given the field-derived constants from Table 2, the amount of amorphous iron oxyhydroxide (sorbant), the amount of sorbed metal and the ambient pH, one can estimate the concentration of the free metal ion,  $M^{z+}$ , in sediment interstitial water at equilibrium with the sediment (equation 6).

$$[M^{z+}] = \frac{\{Fe - OM\}}{K_M \{Fe - ox\}} \quad (6)$$

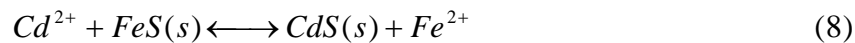
In a more recent publication (Tessier et al. 1993), the model for Cd sorption has been generalized to include both Fe-ox and sedimentary organic matter as sorbants:

$$[Cd^{2+}] = \frac{\{Cd\}_T [H^+]^{1.78}}{10^{-1.22} \{Fe-ox\} [H^+]^{0.96} + 10^{-2.38} \{OM\} [H^+]^{0.82}} \quad (7)$$

where  $\{Cd\}_T$  is the total concentration of non-detrital Cd in the sediment and  $\{OM\}$  is the concentration of sedimentary organic matter.

## 2.2 Anoxic/suboxic sediments

Under anoxic, sulfate-reducing conditions, the partitioning of trace metals between the pore waters and the solid phase is likely to be controlled by precipitation-dissolution reactions involving amorphous sulphides. Evidence supporting this hypothesis has recently been presented by Di Toro and coworkers (1990; 1992). By titrating suspended synthetic iron monosulphide or natural sediments with Cd, these workers demonstrated that the added Cd could react with the amorphous sulphides according to equation (8). Since the solubility product for CdS(s) is lower than that of FeS(s), the equilibrium in equation (8) will lie to the right.



They concluded that under their experimental conditions the concentration of the free  $Cd^{2+}$  ion was controlled not by sorption reactions, nor by reaction with free  $S^{2-}$  in solution, but rather by reaction with solid iron and manganese monosulphides. The reactive sulphide fraction was operationally defined by cold acid extraction of the sediments (one-hour treatment with 0.5 M HCl at room temperature; the forms extracted in this manner are defined as "acid-volatile sulphide", or AVS).

Di Toro et al. (1990; 1992) further hypothesized that since the sulphides formed by Ni, Zn, Pb, Cu and Hg are also less soluble than FeS or MnS, they too should tend to displace Fe or Mn from the AVS fraction. Using the solubility products for metal sulphides ( $K_{sp}$ ; Table 3), one should be able to estimate the free- metal ion concentration in solution.

Table 3: Metal sulphide solubility products (from Di Toro et al. 1992).

Metal sulphide	log $K_{sp}$	log ( $K_{MS}/K_{FeS}$ )
=====	=====	=====
FeS	-22.39	
NiS	-27.98	-5.59
ZnS	-28.39	-6.00
CdS	-32.85	-10.46
PbS	-33.42	-11.03
CuS	-40.94	-18.55
HgS	-57.25	-34.86

### 2.3 Application to *in situ* sediments

The two approaches described above have as a common goal the prediction of dissolved metal concentrations in the pore waters of natural, *in situ* sediments. In effect, pore-water metal concentrations are considered to reflect the metal's chemical potential at the sediment-water interface; changes in this chemical potential should in turn affect the metal's bioavailability. The two approaches differ, however, in their choice of the reactions controlling metal solubility in sediment pore waters. This divergence stems from a different conception of what constitutes "biologically important sediments" - fully oxidized, surficial sediments (where AVS levels should be vanishingly low and  $[M]_i$  should be controlled by sorption reactions) vs. partially oxidized, sub-oxic sediments (where significant AVS levels would be expected to persist, and exchange reactions with the amorphous sulphides would control metal partitioning between the dissolved and solid phases).<sup>2</sup>

Choosing between the two approaches is not as straightforward as might appear at first glance, even for natural sediments (let alone mine tailings!). In the real world, due to spatial heterogeneity at the centimetre scale, the distinction between oxic and anoxic sediment strata is often blurred. Most aerobic benthic organisms survive in sediments that are underlain or even surrounded by completely anaerobic sediments, which constitute a potential source of AVS. Di Toro et al. (1992) suggest that the presence of AVS in the anaerobic layer may be sufficient to reduce the metal activity to which the aerobic benthic animals are exposed, but admit that field data to support this contention are lacking. The question of field validation is considered in detail in section 3.4.

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<sup>2</sup>For a thought-provoking discussion of this point, see Luoma and Davis (1983).

*Depending on which geochemical approach proves more appropriate for deposited mine tailings (i.e., fully oxidized or partially oxidized conditions prevailing at the sediment-water interface), one should be able to use one or the other to predict  $[M]_i$ ; based on the geochemistry of the tailings after diagenesis and the admixture of natural particulate material.*

### **3. INTERACTIONS BETWEEN DISSOLVED TRACE METALS AND AQUATIC ORGANISMS.**

In the previous section, we have considered possible geochemical controls on metal concentrations in pore waters in natural sediments and, by extrapolation, in submerged mine tailings. The present section takes the reasoning one step further (see Table 1) and examines the bioavailability of these dissolved metals<sup>3</sup>.

Much qualitative evidence exists to the effect that the total aqueous concentration of a metal is not a good predictor of its "bioavailability", i.e. that the metal's speciation will greatly affect its availability to aquatic organisms. Note that the term "speciation" is widely used/misused in the trace metal literature (Bernhard et al. 1986); in the present report the term will be used in the passive sense to mean "the distribution or partitioning of a metal among various physico-chemical forms in external medium".

#### **3.1 Background information.**

In much of the early (pre-1975) research on metal-organism interactions, the emphasis was on the target organism and the influence of biological variables (e.g.: life stage; nutrition; age; etc.) rather than on the exposure regime (metal speciation, pH, [Ca], alkalinity, ionic strength, ...). Many of the experiments were simply performed by adding the metal to standard growth medium, with no real appreciation of how this medium might affect the speciation of the metal of interest. There was however a qualitative recognition of the importance of metal "complexation", gained largely from experiments where metals were added to the normal growth medium, with or without various metal-binding ligands.

A major impetus for change occurred early in the 1970's with the popularization of computer codes designed to perform complex chemical equilibrium calculations (e.g., Morel and Morgan 1972). The availability of such codes opened up the possibility of performing bioassays in defined synthetic media, containing only known ligands, and of being able to manipulate the speciation of the metal. A subtle shift of emphasis ensued, from the target organism to the chemistry of the exposure medium; bioassays were performed in defined media, with synthetic ligands (known stability constants, and hence known metal speciation).

In the following years (1975-85) a convincing body of evidence was developed to support the tenet that the biological response elicited by a dissolved metal is usually a function of the free metal ion concentration,  $M^{Z+}(H_2O)_n$ , which in turn is determined not only by the total dissolved metal concentration but also by the concentration and nature of the ligands present. To rationalize these experimental observations and explain what was perceived as "the universal importance of free metal ion activities in determining the uptake, nutrition and toxicity of all cationic trace

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<sup>3</sup>Much of the discussion in this section has been extracted from a chapter in a book that will be published later this year or early in 1994 (Campbell 1993).

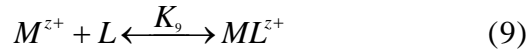
metals", Morel (1983) formulated the Free-Ion Activity Model (FIAM) for metal-organism interactions.

### 3.2 Formulation of the FIAM

To elicit a biological response from a target organism or to accumulate within this organism, a metal must first interact with/traverse a cell membrane. This interaction of the metal with the cell surface, involving either the free metal ion ( $M^{Z+}$ ) or a metal complex ( $ML^{Z+}$ ) as the reactive species, can be represented in terms of the formation of M-X-cell surface complexes, where X-cell = a cellular ligand present at the cell surface.

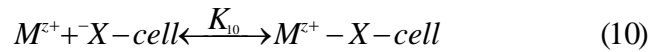
In the simplest case, where the free metal ion is the species reacting at the cell surface, one can envisage the following reactions (where for simplicity charges on the ligand are not shown):

#### solution equilibria



$$K_9 = \frac{[ML^{z+}]}{[M^{z+}][L]} \quad (9a)$$

#### surface reaction of $M^{Z+}$



$$[M^{z+} - X-cell] = K_{10}[^-X-cell][M^{z+}] \quad (10a)$$

where  $K_9$  and  $K_{10}$  are apparent (concentration) equilibrium constants. The biological response is assumed to be proportional to the concentration of the surface complex,  $[M^{Z+}-X-cell]$  (see below). Provided the concentration of free X-cell sites remains approximately constant, equation (10a) indicates that the biological response will vary as a function of  $[M^{Z+}]$ . A similar situation prevails if a metal complex (ML) is the species reacting at the cell surface, provided that the reaction proceeds by ligand-exchange (Campbell 1993).

Possible mechanistic links between the formation of a surface complex, M-X-cell, and the initiation of a biological effect have been suggested by several workers (Morel 1983; Pagenkopf 1983). If X-cell represents a physiologically active site at the cell surface, then the binding of metal M might induce a direct biological response (e.g.: fish gills - Pagenkopf 1983). Alternatively, if X-cell corresponds to a transport site that allows metal M to traverse the cell membrane and enter the cytosol, then binding at the surface site would simply precede transport into the cell (i.e., the actual reaction of M with the metabolically sensitive site would occur intracellularly, following transport - Morel 1983). In a variation of this scenario, X-cell might correspond to a transport site normally used by an essential

micronutrient; binding at the cell surface site by metal M would then inhibit the supply of the essential element and induce nutrient deficiency (e.g.: phytoplankton - Sunda and Huntsman 1983 (Mn,Cu); Harrison and Morel 1983 (Fe,Cd)).

A number of key assumptions underlie the Free-Ion Activity Model, some obvious and others rather more subtle:

- (1) The key interaction of a metal with a living organism involves the plasma membrane, which is impermeable to the free metal ion,  $M^{Z+}$ , and to its (hydrophilic) complexes,  $ML^{\pm}$ .
- (2) The interaction of the metal with the plasma membrane can be described as a surface complexation reaction, forming  $M^{Z+}$ -X-cell (equation 10). The biological response, whether it be metal uptake, nutrition or toxicity, is proportional to the concentration of this surface complex.
- (3) Metal transport in solution, towards the membrane, and the subsequent surface complexation reaction occur rapidly, such that a (pseudo-)equilibrium is established between metal species in solution and those at the biological surface ("rapid" = faster than metal uptake, faster than the expression of the biological response).
- (4) In the range of metal concentrations of toxicological interest, variations in  $[M\text{-X-cell}]$  follow those of  $[M^{Z+}]$  in solution (equation 10a).

### **3.3 Applicability of the FIAM - studies with dissolved metals**

In principle the Free-Ion Activity Model should apply to aquatic organisms that do not assimilate particulate material (e.g., rooted aquatic plants), and to organisms that do assimilate particulate material but for which the dissolved phase remains the primary vector for metal uptake (e.g., fish).<sup>4</sup>

A considerable body of experimental evidence has accumulated in support of the Free-Ion Activity Model - examples of such experiments have recently been critically reviewed by Campbell (1993). Several points of general interest can be extracted from this review.

#### organisms studied

Studies on marine and freshwater phytoplankton outnumber all others. This distribution presumably reflects the sensitivity of algae to trace metals (Morel 1983) and the relative ease with which they can be studied in the laboratory (defined inorganic growth media; rapid response). References to invertebrates are also frequent, with a distinct bias towards marine species. In contrast, the few fish studies were all performed with freshwater species.

Various "end-points" have been studied in the bioassays, covering a spectrum from highly specific (e.g. metal accumulation *per se*: surface adsorption; absorption; subcellular distribution) to more holistic (e.g., growth, motility, mortality).

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<sup>4</sup>One could also argue that even in the case where ingested particulate material constitutes the primary vector for metal uptake, provided the particulate material being ingested is itself in equilibrium with the ambient water, then the response of the consuming organism may still depend indirectly on the free-metal ion concentration in the ambient water.



#### experimental conditions (metals/ligands)

Almost without exception, the experiments designed to test the FIAM have been performed at a fixed pH, with divalent trace metals (Cu, Cd, Ni, Pb, Zn), in artificial (inorganic) media or in filtered seawater, and in the presence of known quantities of synthetic ligands such as EDTA or NTA. These ligands act as metal buffers - by manipulating  $[M]_T$  and/or  $[L]_T$ , one can adjust the free-ion concentration,  $[M^{Z+}]$ , over the desired range. Perhaps fortuitously, these ligands form hydrophilic complexes with the metals of interest, towards which biological membranes are virtually impermeable.

Copper has been studied far more extensively than any other metal. Relevant citations decrease in the order  $Cu > Cd > Zn \gg$  all others.

The major conclusions of the review are summarized below.

#### studies performed in the *absence* of natural dissolved organic matter (DOM)

- In experiments performed at constant pH and water hardness, in the presence of synthetic ligands forming hydrophilic metal complexes ( $ML^\pm$ ), the biological response consistently varies as a function of the concentration of the free metal ion, as predicted by the FIAM (52 of 59 cases reviewed).
- Documented examples of experiments where changes in metal bioavailability do not conform to the FIAM are relatively few in number, and most of these can be explained as cases where the metal forms a neutral, lipophilic complex ( $ML^\circ$ ) to which biological membranes are permeable.
- For studies performed with freshwater organisms under conditions where the pH and/or the water hardness was varied, it is clear that one must consider potential competition for the metal binding site,  $-X$ -cell, by the hydrogen ion,  $H^+$ , and by the hardness cations,  $Ca^{2+}$  and  $Mg^{2+}$ . These effects were explicitly included in Morel's original formulation of the FIAM (Morel 1983), but since most of the early work was done in seawater variations of  $[H^+]$  and  $[Ca^{2+}]$  were of relatively little concern. The influence of the hydrogen ion on metal-organism interactions has been reviewed by Campbell and Stokes (1985).

#### studies performed in the *presence* of natural DOM

- Particularly striking is the scarcity of studies suitable for testing the applicability of the FIAM in the presence of DOM. There are numerous reports in the literature of the effects of DOM on metal bioavailability, but virtually all of these studies are qualitative in nature (i.e., metal speciation is undefined).
- The few quantitative studies that do exist are more-or-less evenly divided between examples that conform to the predictions FIAM and others that appear to be in contradiction - i.e., unlike the studies performed in the absence of DOM, no consensus is evident for the experiments run in the presence of DOM. The applicability of the FIAM in natural waters in the presence of natural DOM remains to be demonstrated (Campbell 1993).

### 3.4 Applicability of the FIAM - studies with sediments

All of the studies considered in the preceding section were carried out in the absence of sediments (water-only exposures); in the present section we return to our area of primary concern, the sediment-water interface, and consider several studies that pertain to simultaneous water-sediment exposures. Two types of study have been carried out: (1) laboratory bioassays on spiked sediments (Di Toro et al. 1990; 1992; Carlson et al. 1991) or on natural sediments collected from known contaminated sites (Ankley et al. 1991; 1993); and (2) field surveys of indigenous benthic organisms (Campbell and Tessier 1989, 1991; Tessier and Campbell 1990; Couillard et al. 1993; Tessier et al. 1993). *Both approaches support the general idea that benthic organisms respond to the free-metal ion concentration at the sediment-water interface.*

#### 3.4.1 Laboratory bioassays

Under the auspices of the U.S. Environmental Protection Agency, a major research effort is currently underway to define criteria for assessing contaminated sediments (Adams et al. 1992; Burton and Scott 1992). One of the avenues being explored is the so-called "Equilibrium Partitioning" (EP) approach, which assumes that the partitioning of contaminants between sediments and pore water can be described on the basis of chemical equilibria - i.e., the concentration of the chemical present in either phase can be estimated by measuring the concentration present in the other phase. The EP approach has proved successful for non-ionic organic contaminants, where the concentrations of the contaminant in the sediment pore water and in the bulk sediment are related by  $K_{oc}$ , the organic-carbon normalized sediment-water partitioning coefficient (Di Toro et al. 1991); attempts to extend this approach to metals are described below.

The goal of the EP approach as applied to metals is to be able to predict the (free-)metal concentration in the sediment pore water. As outlined earlier (section 2.2), the EPA-sponsored researchers have assumed that this concentration is controlled by precipitation reactions with amorphous sulphides (AVS). Since in the presence of excess AVS the concentrations of free metal should be vanishingly low (Di Toro et al. 1990, 1992), these workers have hypothesized that provided the molar ratio of  $\{SEM\}/\{AVS\}$  is less than unity, the metal should not exhibit any toxicity ( $SEM =$  "simultaneously extracted metal" = metal extracted by the same cold acid reagent used to determine the amorphous volatile sulphide fraction). The corollary is that if the  $\{SEM\}/\{AVS\}$  ratio *exceeds* 1, the metal should be toxic.

In a number of recent papers the EPA-sponsored research team (based at Manhattan College and at the US EPA Duluth laboratories) has tested this hypothesis in the laboratory, using various benthic bioassay organisms. Of the five studies summarized in Table 4, three were performed on spiked sediments (#1 to 3) and two on "naturally" contaminated sediments that had been collected along a contamination gradient (#4 and #5). All the studies share a number of common features: the sediments were collected as a grab sample, i.e. with no attempt to distinguish between the oxic or sub-oxic strata, and were then homogenized and analyzed for  $\{AVS\}$  and for the simultaneously extracted metals,  $\{SEM\}$ . In experiments #1 to #3, the  $\{SEM\}/\{AVS\}$  ratio was varied by spiking the sediment with increasing amounts of the test metal. After a short "equilibration" period the sediments were placed in small containers and allowed to settle (normally 24 h). The test organisms were then introduced

and their survival monitored over the next 4-10 days. In most of these experiments the sediments were recovered at the end of the bioassay, re-homogenized, and re-analyzed for {SEM} and {AVS}. Virtually the same protocol was followed for the experiments with naturally contaminated sediments (#4 and #5), except that no additional metal was added to the sediments; {SEM}/{AVS} ratios were determined on the original sediment and again at the end of the bioassay, both times on homogenized samples.

The bioassays run with **cadmium** or **nickel** conformed to the AVS model (Table 4: experiments #1 to #4): both for spiked sediments and for the naturally contaminated sediments, no significant mortality occurred relative to controls if the molar concentration of simultaneously extracted cadmium or nickel was less than the molar concentration of acid volatile sulphide (AVS) in the sediment. Mortality began to occur when the {SEM}/{AVS} ratio reached unity, and total mortality was observed when the ratio exceeded ~3. The results for **copper** were, however, markedly different (Table 4: experiment #5). Normalization of sediment copper concentrations to AVS accurately predicted that sediments were non-toxic when the {Cu}/{AVS} ratio was less than one, but *toxicity was frequently absent in samples with {Cu}/{AVS} ratios significantly greater than unity*. The authors determined pore-water copper concentrations in these experiments and showed that  $[Cu]_i$  still was an excellent predictor of amphipod mortality - toxicity curves for water-only exposure and for pore-water exposure were superimposed (Ankley et al. 1993). The authors concluded that "AVS is not the appropriate partitioning phase for predicting copper bioavailability in freshwater sediments".

Relationships between metal bioaccumulation and {SEM}/{AVS} ratios have also been investigated, but the data base is more limited than for organism mortality (Table 4, column 3). In experiment #2 (*Lumbriculus variegatus*; *Helisoma sp.*) whole-body Cd concentrations were measured in worms and snails that survived to the end of the bioassay (i.e., for sublethal conditions, where the majority of the test organisms survived). Cadmium bioaccumulation tended to increase over the range of experimental {Cd}/{AVS} values from 0.05 to 3, with separate and distinct plots for each sediment. In addition, contrary to what would have been predicted, there was no distinct threshold effect when the ratio {Cd}/{AVS} ratio exceeded 1.0 (Carlson et al. 1991). In contrast to these results, in experiment #4 (*L. variegatus*) negligible bioaccumulation of Cd or Ni occurred at {SEM}/{AVS} ratios less than one. For ratios greater than one, the magnitude by which {SEM}/{AVS} exceeded unity

Table 4: Laboratory tests of the hypothesis that acid volatile sulphide (AVS) controls the toxicity of metals in sediments.

<u>Metal</u>	<u>Organism</u>	<u>Response</u>	<u>Geochemical Predictor</u>	<u>Sediment</u>	<u>Reference</u>
1. Cd	Marine amphipods <i>Ampelisca abdita</i> ; <i>Rhepoxynius hudsonii</i>	Mortality (96 h)	ratio {added Cd}/{AVS}	spiked marine sediments	Di Toro et al. 1990
2. Cd	Freshwater oligochaete <i>Lumbriculus variegatus</i> ; snail <i>Helisoma sp.</i>	Mortality (10d)  Bioaccumulation	ratio {added Cd}/{AVS}	spiked freshwater sediments {SEM}/{AVS} varied from 10 to 0.1)	Carlson et al. 1991
3. Cd, Ni	Marine amphipods <i>Ampelisca abdita</i> ; <i>Rhepoxynius hudsonii</i>  Freshwater oligochaete <i>Lumbriculus variegatus</i> ; snail <i>Helisoma sp.</i>	Mortality	ratio {added M}/{AVS}	spiked freshwater or marine sediments	Di Toro et al. 1992
4. Cd, Ni	Freshwater amphipod <i>Hyalella azteca</i> ; oligochaete <i>Lumbriculus variegatus</i>	Mortality (10d)  Bioaccumulation	ratio {SEM}/{AVS}	estuarine sediments sampled along contamination gradient; {SEM}/{AVS} varied from 0.1 to 220	Ankley et al. 1991
5. Cu	Freshwater amphipod <i>Hyalella azteca</i>	Mortality (10d)	ratio {SEM}/{AVS}	freshwater sediments sampled along contamination gradient; {SEM}/{AVS} varied from 0.2 to 68	Ankley et al. 1993

was however an unreliable predictor of bioaccumulation. As pointed out by the Ankley et al. (1991), this is not surprising since the {SEM}/{AVS} ratio is dimensionless; high ratios can

in principle occur in sediments with relatively low or relatively high metal contamination. The authors sought to improve the prediction of bioaccumulation by taking into account the "excess" metal in the sediment, i.e. the difference {SEM}-{AVS}, but did not find any "definable relationship".

In designing their sediment bioassays, the US EPA-sponsored researchers have emphasized the importance of establishing and maintaining reproducible experimental conditions. While understandable and even commendable, this stress on reproducibility has unfortunately resulted in a certain artificiality - *it is not at all clear that the results of the bioassays presented in Table 4 can be extrapolated from the laboratory to the field.*

This criticism of the bioassay approach bears on three related points: sediment diagenesis, bioturbation, and microhabitats. As pointed out in the first paper in the series (Di Toro et al. 1990), the normal method of preparing sediments for bioassays is to produce a uniform mixture of sediment and pore water by thorough mixing. For such systems the AVS and the metals will be uniformly distributed and the concentrations to be used for calculating the {SEM}/{AVS} ratio are relatively unambiguous. However, as soon as the sediment is placed in the test container, even before the bioassay organisms are introduced, *it begins to change!* Under the influence of the indigenous sediment microorganisms, a redox cline rapidly develops across the sediment-water interface. Vertical gradients are established in the pore water, for such variables as dissolved oxygen, iron (II), manganese(II), sulphate and sulphide, and in the solid phase for such key phases as iron(III) and Mn(IV) oxyhydroxides, and iron(II) plus manganese(II) sulphides (these latter two being major contributors to the AVS fraction - Di Toro et al. 1990). Thus, even in the absence of benthic invertebrates, the distribution of SEM and AVS in settled sediments would be expected to vary vertically and temporally (Herlihy et al. 1988; Nriagu and Soon 1985)<sup>5</sup>.

The introduction of test organisms into the experimental systems further complicates the system. In reworking the sediment, either to create their own microhabitat or in the course of their normal feeding/excretion behaviour, benthic animals will exert an influence on the diagenetic reactions described above. The influence of this "bioturbation" on sediment chemistry was ignored in all but the most recent of the bioassay experiments summarized in Table 4 (i.e., the determinations of {SEM} and {AVS} were performed on sediments collected from parallel control containers without test organisms). Note, too, that benthic invertebrates can create their own microhabitat within the sediment (Aller 1978; Aller and Yingst 1978; Krantzberg 1988). The activity of the animals (burrowing, feeding) creates micro-scale variations in sediment chemistry, which will be superimposed on the general vertical redox-cline. For example, the physical exchange of water between the sediment pore-water compartment, the burrow and the overlying water column will tend to favour the diffusion of oxygen into an otherwise anoxic environment.

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<sup>5</sup> These reactions occur sufficiently rapidly to affect sediment chemistry during the course of a 4 to 10 day bioassay. Indeed, marked changes in {SEM}/{AVS} ratios are often noted when samples collected at the beginning and end of the bioassay are compared (e.g., Di Toro et al. 1990; Ankley et al. 1993). Note that these differences cannot be attributed to a sampling artifact - the sediments are normally analyzed at the end of the bioassay after re-homogenization.

It is clear from the preceding discussion that under the combined influence of sediment microorganisms and benthic macrofauna, SEM and AVS concentrations in sediments will vary temporally and vertically. Given this variability, it is difficult to decide which SEM and AVS concentrations should be used to evaluate the potential toxicity of the metals in sediments. Concentrations of SEM and AVS can be measured reproducibly on a homogeneous sediment suspension .... but how do these values relate to the true exposure conditions experienced by benthic organisms *in situ*?

It should be emphasized that the preceding critical analysis applies to the AVS approach, as tested in the laboratory, but does not imply a rejection of the Equilibrium Partitioning paradigm *per se*. On the contrary, we would argue that the EP model (with its emphasis on the prediction of the free-metal ion concentration,  $[M]_i$ , in the interstitial water - *see Table 1*) constitutes a valid conceptual framework - what remains to be determined is the nature of the geochemical phases/reactions that are involved in controlling  $[M]_i$ . Normalization of metal concentrations with respect to AVS is, of course, invalid if  $\{AVS\}$  is zero, as would be the case in a fully oxidized sediment. For sediments with trace amounts of AVS, Di Toro et al. (1990) suggest that it is likely that "other phases" (e.g., sedimentary organic matter; iron oxyhydroxides) would be important. Indeed, in their most recent laboratory experiments with copper, Ankley et al. (1993) conclude that strong binding phases other than AVS exist for copper in freshwater sediments, and suggest that this additional binding capacity may be correlated with sedimentary organic matter.

All the studies discussed to this point have been carried out in the laboratory on "manipulated" sediments. Field studies on *in situ* sediments and indigenous benthic organisms might be expected to yield a clearer indication as to which geochemical phases/reactions are involved in controlling metal bioavailability at the sediment-water interface. Such studies are the subject of the following section.

### 3.4.2 Field Studies

As an alternative to the laboratory bioassay approach described in the preceding section, one could in principle also study metal-contaminated sediments *in situ*. The literature is however replete with unsuccessful examples of this approach, notably where researchers have sampled along a contamination gradient and have sought relationships between metal levels in the indigenous benthic organisms and total metal concentrations in the host sediments. More recently, as the link between metal speciation and metal bioavailability has become better recognized, attempts have been made to refine the geochemical approach used to define the contamination gradient. In particular, several recent papers have used a geochemical modeling approach to estimate the free-metal ion concentration at the sediment-water interface, and have then defined the contamination gradient in terms of  $[M^{Z+}]$ . The present section focuses on such investigations.

All the studies considered in this section were performed in the field, at sites chosen to represent a metal contamination gradient (Table 5). Surficial oxic sediment samples were collected at each site and analyzed to define and quantify the contamination gradient from a

geochemical point of view. Similarly, to evaluate the bioavailability of the sediment-associated metals, indigenous benthic organisms were collected and analyzed. The common goal of these studies was thus to relate the metal concentrations observed in the benthic organisms to the geochemistry of the host sediments. In each case the geochemical gradient has been defined in terms of either the free-metal ion (as estimated from sediment-water equilibria - section 2.1) or the ratio of sorbed metal to sorbant. Provided that the ambient pH varies little along the contamination gradient, this latter ratio can be taken as a surrogate measure of the free-metal ion concentration (NRCC 1988; Tessier and Campbell 1990; Tessier et al. 1993).

Pathways for metal accumulation differ for the two types of benthic organisms represented in Table 5. Rooted aquatic plants live in intimate contact with the sediments but can only take up metals from the dissolved phase (sediment interstitial water and/or overlying water column). In the single study relating to this class of organism, regression analysis was used to examine the relationship between metal concentrations in the plant roots/rhizome ( $[M]_r$ )<sup>6</sup> and metal concentrations at the sediment-water interface. Since the pH varied little over the limited study region,  $[H^+]^x$  was dropped from equation (5) and relationships were sought between the metal levels in the plant (dependent variable) and the ratio  $\{Fe-OM\}/\{Fe-ox\}$  in the host sediment. The usefulness of this ratio as a predictor varied among metals (Cu > Pb, Zn) and among plants (*Potamogeton richardsonii* > *Eleocharis smallii*, *Nuphar variegatum*, *Sparganium americanum* > *Glyceria borealis*) - see Campbell and Tessier (1991). These preliminary results suggested that the pondweed *P. richardsonii* may prove to be useful as a biomonitor species for sediment-associated metals. However, to confirm the potential of this species and the usefulness of the expression  $\{Fe-OM\} [H^+]^x / \{Fe-ox\}$  as a surrogate for  $[M^{Z+}]$ , it would be necessary to extend the study to a wider geological setting.

Benthic invertebrates are directly exposed to sediment-bound metals and may accumulate metals from the ambient water and/or from ingested sediment (Luoma 1983; NRCC 1988). If water is the more important exposure vector,  $[M]_{benthos}$  should be correlated with the free-metal ion concentration in the interstitial water ( $[M^{Z+}]$  or its surrogate). This hypothesis has been tested for a variety of filter- and deposit-feeding invertebrates (Table 5). The general experimental approach has involved field studies at lacustrine or estuarine sites located along a spatial contamination gradient. Measured biological variables have included metal concentrations in the indigenous organisms and, in one case, metallothionein levels. Regression analysis was used to examine relationships between the biological response and either the free-metal ion (as estimated from sediment-water equilibria - section 2.1) or the ratio of sorbed metal to sorbant. Relationships between the biological response and these

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<sup>6</sup> For submerged rooted species in a field setting, the least ambiguous indicator of metal bioavailability in the sediments will be the metal concentrations in the underground parts of the plants (Schierup and Larsen 1981; NRCC 1988).

Table 5: Relationships between metal bioavailability, as sensed by indigenous benthic organisms, and geochemical estimates of the free-metal ion concentration present at the oxic sediment-water interface.

<u>Metal</u>	<u>Organism</u>	<u>Response</u>	<u>Geochemical predictor</u>	<u>Site<sup>a</sup></u>	<u>Reference</u>
1. Cu Pb	Rooted freshwater aquatic plants, e.g. <i>Potamogeton richardsonii</i>	Metal bioaccumulation	{Fe-OM}/{Fe-ox}	Rouyn-Noranda (N=10; r <sup>2</sup> =0.72 for Cu r <sup>2</sup> =0.62 for Pb)	Campbell and Tessier 1991
2. Cd	Filter-feeding freshwater mollusk <i>Anodonta grandis</i>	Metal bioaccumulation	[Cd <sup>2+</sup> ], as estimated from oxic sediment-water equilibria	Rouyn-Noranda; Chibougamau; Eastern Townships, Quebec; Sudbury; Muskoka (N=19; r <sup>2</sup> =0.82)	Tessier et al. 1993
3. Cd	Filter-feeding freshwater mollusk <i>Anodonta grandis</i>	Metallothionein induction	[Cd <sup>2+</sup> ], as estimated from oxic sediment-water equilibria	Rouyn-Noranda (N=11; r <sup>2</sup> =0.56)	Couillard et al. 1993
4. Cu	Filter-feeding freshwater mollusk <i>Elliptio complanata</i>	Metal bioaccumulation	{Fe-OCu}/{Fe-ox}, both extracted with NH <sub>2</sub> OH•HCl	Rouyn-Noranda (N=8; r <sup>2</sup> =0.95)	Tessier et al. 1984
5. Pb	Estuarine deposit feeder <i>Scrobicularia plana</i>	Metal bioaccumulation	{Fe-OPb}/{Fe-ox}, both extracted with HCl	UK estuaries (N=37; r <sup>2</sup> =0.88)	Luoma and Bryan 1978
6. Hg	Estuarine deposit feeder <i>Scrobicularia plana</i>	Metal bioaccumulation	{Hg}/{OM}, Hg extracted with HNO <sub>3</sub> , organic matter determined by loss on ignition	UK estuaries (N=78; r <sup>2</sup> =0.63)	Langston 1982
7. As	Estuarine deposit feeder <i>Scrobicularia plana</i>	Metal bioaccumulation	{Fe-OAs}/{Fe-ox}, both extracted with HCl	UK estuaries (N=75; r <sup>2</sup> =0.93)	Langston 1980

<sup>a</sup> N = number of field sites.



geochemical predictors were consistently highly significant (Table 5). In contrast, correlations with total metal concentrations in the sediments were consistently weaker and often statistically insignificant.

The approach to metal bioavailability described in this section is based on the Free-Ion Activity Model (FIAM). It draws on surface complexation (SC) concepts (sorption) to predict the free-metal ion concentrations that should prevail in equilibrium with oxic sediments, and then assumes that the biological response should vary as a function of this concentration,  $[M^{Z+}]$ . The success of the approach under field conditions, as demonstrated in Table 5, suggests that the underlying concepts may well be of general applicability. It should be pointed out, however, that all the organisms represented in Table 5 live at the sediment water interface (as opposed to within the sediment, e.g. in burrows, where AVS might be expected to play a more important role). It remains to be seen if the FIAM-SC approach also applies to infaunal organisms whose behaviour brings them into contact with sub-oxic sediments.

#### **4. INTERACTIONS BETWEEN PARTICULATE TRACE METALS AND AQUATIC ORGANISMS**

Having dealt with dissolved metals (geochemical controls; bioavailability) in the previous two sections, we now focus on those metals that remain in particulate form, i.e. the direct route of exposure. The reasoning described below applies to organisms for which particulate material constitutes an important vector for metal uptake. We describe possible links between digestive processes in benthic organisms and the fate of ingested sediment-bound metals in the intestinal tract, and review the results of laboratory experiments designed to determine the availability of metals present in different forms in the "food".

##### **4.1 Background information**

Benthic organisms that ingest particles tend to select and ingest the smaller and lighter particles in their environment. This nutritional strategy is "designed" to maximize the ingestion of organic carbon in the form of attached bacteria or surface-bound organic coatings, but it also results in the ingestion of fine-grained particles that tend to be enriched in metals<sup>7</sup>.

Assimilation of particle-bound metals will normally involve their conversion from particulate to dissolved form in the gut, followed by their facilitated diffusion across the intestinal membrane (Luoma 1983). Digestive processes and the chemical conditions prevailing within the intestinal tract thus assume considerable importance (e.g., pH, digestion times, redox status). **Digestive pH** values tend to vary for different taxa and, within the gut of a complex organism, may vary longitudinally. For lower trophic level invertebrates, such as those found at the sediment-water interface, circumneutral pH values are the norm - pH 5 is the lowest reported for the digestive tract of suspension feeders such as oysters (Owen 1966), whereas the digestive pH of most deposit-

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<sup>7</sup> For example, Tessier et al. (1984) recovered the gut contents from two freshwater molluscs (*Elliptio complanata* and *Anodonta grandis*; discussed in section 3.4.2), and showed that more than 90% of the particles were smaller than 80  $\mu$  m.

feeding organisms is in the range 6-7 (Luoma 1983; NRCC 1988). More extreme pH conditions are observed in upper trophic level organisms. Intuitively, acidic conditions within the gut might be expected to favour metal desorption and dissolution, leading to higher concentrations of dissolved metals in the lumen. Counteracting this effect, however, will be the tendency of the hydrogen-ion,  $H^+$ , to compete with the dissolved metal for uptake sites on the intestinal membrane (Luoma 1983; Campbell and Stokes 1985). Indeed, the link between (low) digestive pH and (enhanced) metal uptake is weak - it would appear that while low pH may indeed extract more metal from the ingested particles, the membrane carriers in such an environment are less efficient in complexing the metal for transport (Luoma 1983).

The **residence time** of the sediments in the digestive tract will also be of importance, since the desorption/dissolution processes involved in the digestive process may be slow - longer digestive times would tend to favour more complete extraction of the metal. Extended residence times in the gut might also affect the **redox status** of the ingested sediment. Microorganisms associated with the ingested sediment will continue to consume oxygen - given sufficient time an originally oxic sediment may well find itself in an anaerobic environment, with obvious consequences for the amorphous Fe- and Mn-oxyhydroxides and their associated metals.

Metal uptake may also occur by **endocytosis** in some benthic invertebrates, but the quantitative importance of this pathway is unknown. Endocytosis is the engulfment of a (metal-bearing) particle by the epithelial membrane, which then pinches off and forms a membrane-limited vesicle within the cell. In a variation of this mechanism, specific amoebocytes and/or digestive vesicles may engulf the particles outside the cell membrane, e.g. in the lumen of the digestive tract or outside the gills, and then move back into the tissues carrying their particulate burden (George et al. 1978; Luoma 1983). Note that although a metal may find itself "within" a tissue as a result of endocytosis, strictly speaking it has not been truly "assimilated" since it is still separated from the cytosol by the original vesicle membrane. Digestive processes within the vesicle might be expected to result in the desorption/solubilization of the metal and its subsequent movement from the vesicle into the cytosol, as discussed above for the intestinal tract. Although such a scenario seems plausible, it remains speculative - little is known about the chemical conditions within digestive vesicles or amoebocytes.

#### 4.2 Laboratory feeding experiments

Having described the conditions likely to prevail in the digestive tract of a typical benthic invertebrate, we can now ask the obvious question: do different metal forms behave differently in this internal environment? One might formulate the same question in a more general manner: is the bioavailability of sediment-bound metals affected by their partitioning among various solid phases?

To answer these questions one could in principle study metal bioaccumulation from different sediment phases, and indeed several such feeding experiments have been performed. The general approach in these experiments has been to (radio)label various "model" substrates, chosen to represent geochemical phases known to be present in natural sediments (e.g., organic detritus; amorphous Fe(III) or Mn(IV) oxyhydroxides; synthetic calcite; biogenic carbonate), and then to offer these substrates as "food" to a sediment-ingesting organism. Accumulation of the labeled metal within the test organism is then taken as evidence of the metal's "bioavailability" (Luoma and Jenne 1976, 1977; Harvey and Luoma 1985a,b).

Though simple and direct, this approach is not without its drawbacks (Luoma 1983). The most important of these is related to the tendency of the (radio)label to re-equilibrate with the solution phase. It is relatively straight-forward to spike a model substrate with radio-labeled metal. However, as soon as the solid phase is recovered and placed in a "clean" aqueous system, the radio-label will tend to redistribute between the model sediment and the aqueous phase. It follows that the test organism will be exposed not only to the particulate metal, but also to metal in solution. *To evaluate the bioavailability of the particulate metal, it will be necessary to correct for any "accidental" uptake from solution.* Similarly, metal redistribution among sediment phases may occur if one type of labeled phase is mixed with several types of unlabeled particles (Tessier and Campbell 1988). Note too that the retention of undigested radio-labeled sediment in the digestive tract of the test organism may bias the results of whole-body metal analyses (Chapman 1985; Hare et al. 1989).

Luoma and Jenne (1976, 1977) managed to minimize the influence of these factors in their studies on *Macoma balthica*., an estuarine deposit-feeder that ingests both suspended particulates and surficial bottom sediments. In a series of elegant experiments, these authors exposed *M. balthica* to various model substrates, each of which had previously been radio-labeled with  $^{110m}\text{Ag}$ ,  $^{60}\text{Co}$ ,  $^{65}\text{Zn}$  or  $^{109}\text{Cd}$ . For each experiment a thin layer of labeled sediment of a single type was placed in an aquarium and covered with sea water. Eight clams were placed in the aquarium and allowed to feed on the sediments. To correct for uptake from solution, four clams were enclosed individually in dialysis bags and placed in the aquarium - the dialysis bag separated the enclosed animals from the sediment, but allowed exposure of both groups of animals to the same concentration of dissolved metal. The experiments were run for 14 d and at various intervals the organisms were removed from their microcosm, counted for radiolabel (non-destructively) and returned to their container. Nuclide concentrations in enclosed animals were subtracted from those in feeding animals to obtain an estimate of uptake from ingestion alone. Corrections were also applied to account for the presence of undigested radio-labeled substrate in the clams' digestive tract.

Significant bioaccumulation by ingestion alone was observed in the Jenne and Luoma experiments, but the *efficiency of uptake varied greatly among different sediment types* (Table 6). For example, uptake of silver varied over three orders of magnitude when normalized for the concentration of radionuclide in each substrate: uptake from calcite or Mn(IV) oxide was much greater than from the other model sediments (amorphous Fe(III) oxide; organic detritus). With the same suite of substrates, uptake of zinc varied over two orders of magnitude; in this case, uptake from biogenic carbonates and organic detritus was greater than from the Fe or Mn oxides. This difference between silver and zinc illustrates an important general conclusion from these feeding experiments, namely that *relative metal availability from a given sink varied from metal to metal*<sup>8</sup>. The differences in metal availability were inversely related to the strength of metal binding to the particulates - sediments that exhibited the highest affinity for the metal (i.e., that released the least amount of metal back into solution) were also the substrates from which metal bioavailability was the lowest. Within the same sediment type (e.g., Fe(III) oxide), metal bioavailability from ingested particles appeared to be sensitive to the degree of crystallinity of the model sediment - uptake of radiolabel from freshly precipitated  $\text{Fe}_2\text{O}_3$  (3 h) was much greater than from slightly aged oxhydroxides (16-24 h).

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<sup>8</sup> In other words, one cannot simply generalize that "metals" are more available from phase A (e.g., organic detritus) than from phase Z (e.g., Mn(IV) oxyhydroxide) - while the sequence  $A > B$  may be true for one metal, it will not necessarily hold for the next.

Table 6: Assimilation of sediment-bound metals by the estuarine deposit-feeder, *Macoma balthica* - summary of controlled feeding experiments with radio-labeled (model) sediments.

<u>METAL</u>	<u>RELATIVE BIOAVAILABILITY SEQUENCE</u>	<u>REFERENCE</u>
Cd	uncoated Fe-ox >> coated Fe-ox, organic detritus	Luoma and Jenne 1976
Ag	calcite>Mn-ox >>biogenic CaCO <sub>3</sub> >Fe-ox>detritus	
Zn	biogenic CaCO <sub>3</sub> >detritus>calcite>Fe-ox, Mn-ox	Luoma and Jenne 1977
Co	biogenic CaCO <sub>3</sub> >calcite ~ detritus>Fe-ox>Mn-ox	
Cd	(exopolymer + Fe-ox) > (bacteria + Fe-ox) ~ uncoated Fe-ox natural sediment >> alkaline extracted sediment extracted sediment + exopolymer ~ original sediment	Harvey and Luoma 1985a
Zn	(exopolymer + Fe-ox) slightly> uncoated Fe-ox natural sediment >> alkaline extracted sediment extracted sediment + exopolymer ~ original sediment	

A similar experimental design was employed by Harvey and Luoma (1985a,b) in their more recent studies on *Macoma balthica* - instead of individual dialysis bags, a filter chamber device was used to separate the feeding from the non-feeding clams. Their experiments were designed to quantify the effects of adherent bacteria and bacterial extracellular polymer ("exopolymer") on the uptake of particle-bound metals. Earlier experiments had shown that adherent bacteria were an important food source for *M. balthica*, but that the bacterial exopolymer (used in the process of bacterial adhesion) was not assimilated in the time frame of the feeding experiments. Various radio-labeled test sediments were used (Table 6): amorphous Fe(III) oxyhydroxides (Fe-ox); amorphous Fe(III) oxyhydroxides to which either *Pseudomonas atlantica*, an adherent bacterium, or its exopolymer had been added; unaltered San Francisco Bay sediments; sediments that had been extracted with an alkaline solution to remove the fulvic/humic acid component; extracted sediments that had been re-coated with bacterial exopolymer.

As in the earlier experiments, amounts of metal taken up from a given model sediment varied greatly from one metal to another. For example, ingestion of radio-labeled but uncoated iron oxyhydroxides did not contribute appreciably to uptake of Ag or Cd, but accounted for 89-99% of Zn uptake. Similarly, amounts of metal taken up from ingested sediment were sensitive to the nature of the particle surface. For example, exopolymer adsorbed onto the Fe-ox surface caused an increase

in the availability of particle-bound metals in the order Ag >Cd>Zn. Adherent bacteria had no effect on metal uptake, even though Cd and Zn were taken up from suspensions of bacteria that had been pre-labeled with metal (Harvey and Luoma 1985b).

In the trials with labeled sediments from San Francisco Bay, ingested particles contributed more than 50% of the Cd uptake. In marked contrast, uptake from alkaline-extracted sediments was negligible - interestingly, addition of bacterial exopolymer to the extracted sediments restored the bioavailability of sediment-bound cadmium (note the parallel with the effect noted earlier for amorphous Fe(III) oxyhydroxides amended with exopolymer). A similar but less dramatic effect was noted for zinc.

Harvey and Luoma (1985a) were unable to explain how the alteration of inorganic particles with bacterial exopolymer enhanced the availability of particle-bound Ag, Cd and Zn to *M. balthica*. They discounted the possibility that the exopolymer simply affected the affinity of the particles for the metal, or that feeding rates changed in the presence of coated particles - they suggested instead that the presence of exopolymer may have stimulated the secretion of enzymes capable of competing with Fe-ox for metals in the clam's digestive tract. The observation that adsorbed bacterial exopolymer and NaOH-extractable organics had a similar effect on the availability of Cd and Zn from ingested natural sediments suggests that the organic component of a natural sediment may have a marked influence on the biological availability of metals.

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The most important conclusion from the above studies is that the physicochemical form of a sediment-bound metal definitely affects its availability in the digestive tract of *Macoma balthica*. However, it should be emphasized that all of the studies were performed on the same deposit-feeder, *M. balthica*, and as the authors themselves point out, it would be premature to attempt to generalize these results to all deposit-feeders.

The results of the feeding experiments are admittedly qualitative and thus are not yet very useful from a quantitative or predictive point of view. Nevertheless, there is an interesting parallel between Luoma and Jenne's "affinity" concept (wherein they suggest that the availability of a particle-bound metal will be inversely related to the strength of the metal-particle association - Luoma and Jenne 1976,1977) and the more recent suggestion by Di Toro et al. (1990) that the concentration of the metal in the interstitial water can be taken as a measure of its "chemical potential" in surficial sediments and thus its availability. This inference seems intuitively reasonable, **provided that the chemical environment within the animal's digestive tract is similar to that in its immediate environment** ( i.e., provided the chemical potential of the metal doesn't change drastically on passing from outside environment into the digestive system). In this context, better knowledge of the chemistry of invertebrate digestion would be very useful<sup>9</sup>.

## **5. ECOTOXICOLOGICAL CONSIDERATIONS**

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<sup>9</sup>Reinfelder and Fisher (1991) recently studied the efficiency with which various ingested elements were assimilated by marine calanoid copepods (zooplankton). These authors fed their animals radio-labeled diatoms rather than sediments, but their results are nevertheless pertinent: assimilation efficiencies ranged from 0.9% to 97%, and were directly related to the cytoplasmic content of the diatoms. Reinfelder and Fisher speculate that these zooplankton, with their short gut residence times, have developed a gut lining and digestive strategy that only provide for assimilation of soluble material.

## 5.1 Biochemical indicators of metal-induced stress

### 5.1.1 Background information

Although the present literature report is focused on the subaqueous disposal of reactive mine tailings, it should not be forgotten that even in the absence of such practices freshwater sediments act as an important reservoir for metals of anthropogenic origin. Whether the fluxes of metals to sediments are gravitational or diffusive, the overall result is a marked enhancement of metal concentrations in the upper strata of lake sediments (Livett 1988) - metal concentrations in such systems can attain levels 1000 - 5000 times higher (on a  $\mu\text{g/g}$  fresh weight basis) than those in the overlying water column.

Even in contaminated lakes, the sediment-water interface is normally the habitat for a diverse and often abundant benthic community. The contamination of surficial sediments by metals of anthropogenic origin is thus of potential ecological importance. Two related questions have attracted the attention of researchers and regulators: **(A)** To what extent are the sediment-bound metals bioavailable? **(B)** Are there demonstrable adverse effects on the benthic community, or on the predatory wildlife that depend on this community, that can be attributed to metal contamination? With regard to question **(A)**, potentially toxic metals can attain very high levels in benthic organisms living in contaminated sediments (particularly in the invertebrate community). Considerable progress has been made towards the development of predictive models that relate metal body burdens in benthic invertebrates to the geochemical conditions prevailing in the host sediments (NRCC 1988; see section 3.4.2), and partial responses to question **(A)** are already available. However, as discussed below, benthic invertebrates possess a variety of metal detoxification mechanisms, and metal bioaccumulation *per se* is not necessarily an indication of adverse effects. The response to question **(B)** thus remains unclear.

Traditionally, attempts to define the impacts of contaminants on aquatic ecosystems have involved laboratory experiments under defined conditions (toxicity tests) and, to a lesser extent, field observations on impacted indigenous populations. Extrapolation of laboratory-derived toxicological data to the field is fraught with difficulties (Kimball and Levin 1985; Cairns 1986), as is the unambiguous interpretation of field observations (Ford 1989 - see section 5.2). An alternative and complementary approach involves the use of **biochemical indicators** to monitor the response of individual organisms to toxic chemicals and to provide a measure of ecosystem health.

### 5.1.2 Biochemical Indicators

The biochemical indicator concept is based on the principle that biological effects of toxic chemicals in the environment are initiated by the interaction of the toxic chemical with a biological receptor in a living organism (NRCC 1985; Huggett *et al.* 1992). The assumption is made that effects at the ecosystem level are preceded by chemical reactions in individual organisms, and that concentrations of the contaminant needed to initiate these reactions are lower than those required to provoke a life-threatening situation for the target organism or perceptible degradation of the ecosystem. The detection and quantification of these chemical reactions could then be developed as a sensitive, specific indicator of environmental stress.

A successful biochemical indicator should satisfy a number of criteria (Stegeman *et al.* 1992; Haux and Förlin 1989):

- (i) the indicator should have an early warning capacity, i.e., the biochemical response should be predictive of effects at higher levels of biological organization and should precede them;
- (ii) the indicator should be specific to a particular contaminant or for a class of contaminants;
- (iii) the indicator should respond in a concentration-dependent manner to changes in ambient levels of the contaminant;
- (iv) the basic biology/physiology of the biomonitor organism should be known so that sources of uncontrolled variation (growth and development; reproduction; food sources) can be minimized;
- (v) levels of the indicator should be related to the health or "fitness" status of the organism.

For **metals**, much of the attention in the area of biochemical indicators has focused on metal-binding proteins, in particular on metallothionein (MT) and metallothionein-like compounds. Metallothionein, a low molecular weight, soluble, metal-binding protein or group of proteins, has been identified in mammals, invertebrates, plants and prokaryotes (Roesijadi 1981, 1992; NRCC 1985; Engel and Roesijadi 1987). Much of the early research on the structure and function of MT was performed on mammals, but over the last 10-15 years marine invertebrates have been studied intensively and the role of MT in their metal metabolism has been clarified (Engel and Roesijadi 1987; Roesijadi 1992) - in distinct contrast, freshwater invertebrates have been studied much less intensively. Metallothioneins isolated from marine invertebrates exhibit properties that differ slightly from those of mammalian MT (e.g.: lower cysteine content; presence of low levels of aromatic amino acids; propensity to form dimers) and thus they are often referred to as "metallothionein-like" proteins. In the present context, we shall use metallothionein (MT) in its generic sense.

In marine invertebrates metallothionein has been implicated in the storage, transport and exchange of essential metals (Cu, Zn), and in the detoxification of these and non-essential metals (Ag, Cd, Hg). As a soluble protein present in the cytosol, with a demonstrably high *in*

*in vitro* affinity for such toxic metals as Ag, Cd and Hg, metallothionein is poised to sequester incoming metals and reduce their availability to critical biochemical sites within the cell (Roesijadi 1981). In such a scheme, as applied to (marine) invertebrates, MT would be involved in the initial detoxification of the incoming metal, and would play a role in funneling the metal to such complementary sinks as lysosomes and granules / concretions (Viarengo 1985).

Support for the putative role of MT in metal detoxification is derived from the observation that exposure to elevated concentrations of Cd, Cu, Hg or Zn often induces the synthesis of MT; in the case of Cd and Hg, the toxic metals have been reported to displace the essential metals normally associated with metallothionein. Consistent with such a role is the observation of enhanced metal tolerance associated with the induction of metallothionein. For example, prior exposure of the marine mussel *Mytilus edulis* in the laboratory to copper or cadmium, at concentrations sufficiently high to induce metallothionein-like proteins, conferred increased tolerance to the toxicity of inorganic mercury (Roesijadi and Fellingham 1987). Similar results have been reported for freshwater fish (Klaverkamp et al., 1984; Roch and McCarter 1984a; Klaverkamp and Duncan 1987).

Despite this correlation between acclimation to metal toxicity and induction of MT synthesis, however, metal sequestration by metallothionein may not be a "pure" metal detoxification mechanism. Metallothionein has been reported as a constitutive (Cu,Zn-)protein in organisms collected from pristine sites, and it has been shown to play a central role in the moulting cycle of marine crustaceans (Engel and Brouwer, 1987). In other words, it is not clear that binding of toxic metals to MT is purely protective and is not itself a deleterious event (Petering and Fowler 1986).

### **5.1.3 Metallothionein as a monitoring tool**

Given its molecular properties, and present knowledge of its role in metal uptake, transport, storage and excretion, metallothionein offers considerable potential as a contaminant-specific biochemical indicator of metal exposure and/or stress (Roesijadi 1992; Stegeman et al. 1992). Possible approaches include:

- (i) Direct measurement of [MT] as a simple indicator of prior exposure to toxic metals.

In this case, it is assumed that constitutive levels of MT are low, and that any increase in concentration above these low basal levels is attributable to the induction of MT in response to an influx of toxic metals. Such an approach would in principle furnish a measure of the toxicologically significant intracellular fraction of metals (Olafson et al. 1979).



- (ii) Examination of the relative distribution of toxic metals in cytosolic ligand pools as a means of evaluating metal stress at the biochemical level.

It has been suggested that excessive accumulation of metals beyond the binding capacity of available MT should result in their binding to other intracellular ligands (notably those of high molecular weight, HMW), a phenomenon termed "spillover" - metals bound to these other ligands are considered to be capable of exerting cellular toxicity (Brown and Parsons 1978). In principle this condition could be considered as symptomatic of metal stress and would be amenable to detection.

Field evidence in support of the first approach is slowly accumulating in the ecotoxicological literature. The studies summarized in Table 7 all involved the sampling of indigenous populations of aquatic animals from sites chosen to represent a spatial (metal) contamination gradient - **in all cases the concentrations of metallothionein-like proteins did indeed prove to be higher at the more contaminated sites**. Note, however, that in most cases the contamination gradient was defined only in a qualitative sense (e.g., upstream and downstream from a known point source of metals, or as a function of distance from a base metal smelter) and a true dose-response relation could not be derived. In only two cases (Roch et al. 1982; Couillard et al. 1993) were strict relations demonstrated between tissue metallothionein concentrations and ambient metal levels.

In the studies of organisms living along a contamination gradient, tissue metallothionein concentrations were often positively correlated with tissue cadmium levels (Table 7). This correlation between [MT] and tissue levels of Cd is consistent with the reported potency of Cd in inducing MT biosynthesis in laboratory experiments (e.g, Roesijadi et al. 1988)<sup>10</sup> and suggests that metallothionein levels will be particularly sensitive to increases in ambient cadmium.

In addition to spatial trends in tissue metallothionein concentrations, several workers have demonstrated that MT levels also respond temporally to changes in the degree of metal contamination (Table 8). For example, Roch and McCarter (1984b) kept hatchery-raised rainbow trout in pens at three contaminated sites in the Campbell River drainage system (May-June 1982: S. Buttle Lake, N. Buttle Lake; John Hart Lake) - elevated levels of hepatic metallothionein had developed after four weeks exposure *in situ*. In a companion study, the levels of hepatic metallothionein in the indigenous Buttle Lake trout population were monitored over the period 1981 to 1985, i.e. as the ambient metal levels declined in response to changes in the tailings disposal methods - hepatic MT concentrations decreased over the same period from a high of 269 ( $\pm 23$ ) nmol/g wet wt in August 1981 to a low of 64 ( $\pm 22$ )

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<sup>10</sup>Jones et al. (1988) showed that the relative ability of metals to induce metallothionein synthesis is inversely correlated with their softness parameter,  $\rho$ . A "soft" electron acceptor is characterized by a high polarizability of its outer electronic shell and a tendency to form stable bonds with "soft" ligands, e.g. those containing free thiol groups, RS<sup>-</sup>. Lower values of  $\rho$  correspond to softer ions;  $\rho$  values for Cd<sup>2+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup> are respectively 0.081, 0.104, and 0.115 (Ahrland 1968).

Table 7: Field validation of the use of metallothionein as a biochemical indicator of exposure to bioavailable metals - spatial studies (N = number of sites).

<u>Field site</u>	<u>Sentinel organism</u>	<u>Tissue</u>	<u>Metal gradient</u>	<u>Result</u>	<u>Reference</u>
Campbell River Watershed, British Columbia (N=4)	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	liver	Zn, Cu, (Cd)  defined in terms of dissolved metal, [M] <sub>d</sub>	Hepatic metallothionein increased ~4-fold in the indigenous trout populations along the contamination gradient; [MT] correlated with hepatic Cu or Cd	Roch et al. 1982
Emån River Sweden (N=2)	Perch ( <i>Perca fluviatilis</i> )	liver	Cd  gradient undefined (up- and downstream from point source)	Hepatic metallothionein higher at downstream site;  [MT] correlated with hepatic Cd in individual specimens (N=20)	Olsson and Haux 1986
Flin Flon lakes, Manitoba (N=6)	White sucker ( <i>Catostomus commersoni</i> )	liver, kidney	Cd, Cu, Zn  defined in terms of distance from smelter	Hepatic and renal metallothionein inversely correlated with distance from smelter	Klaverkamp et al. 1991
Rouyn-Noranda lakes, Quebec (N=11)	Freshwater mollusc ( <i>Anodonta grandis</i> )	gills; whole organism	Cd  defined in terms of [Cd <sup>2+</sup> ] at sediment-water interface	Metallothionein increased 2.5- to 4-fold in the indigenous mollusc populations along the contamination gradient; [MT] correlated with tissue Cd	Couillard et al. 1993
Canadian Atlantic coast (N=7)	Various seabirds	kidney	gradient undefined	[MT] and [Cd] correlated in the kidneys of Leach's storm-petrels, Atlantic puffin and herring gull	Elliot et al 1992

Table 8: Field validation of the use of metallothionein as a biochemical indicator of exposure to bioavailable metals - temporal studies.

<u>Field site</u>	<u>Sentinel organism</u>	<u>Tissue</u>	<u>Metal gradient</u>	<u>Result</u>	<u>Reference</u>
Campbell River Watershed, British Columbia (Buttle Lake; John Hart Lake) (Exposure period = 4 weeks)	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	liver	Zn, Cu, (Cd) defined in terms of $[M]_d$	Hepatic metallothionein increased ~3-fold in the trout held for 4 weeks in net pens at three locations contaminated by metals; after 4 weeks exposure <i>in situ</i> , hepatic [MT] was correlated with degree of contamination as measured by $[Zn]_d$	Roch et McCarter 1984b
Campbell River Watershed, British Columbia (Buttle Lake) (Exposure period = 4 years)	Rainbow trout ( <i>Oncorhynchus mykiss</i> )	liver	Zn, Cu, (Cd) defined in terms of $[M]_d$	Hepatic metallothionein decreased ~4-fold in the indigenous trout populations from 1981 to 1985, presumably in response to the decrease in ambient dissolved metal levels.	Deniseger et al. 1990
Rouyn-Noranda, Quebec (Lake Vaudray) (Exposure period = 400 days)	Freshwater mollusc ( <i>Anodonta grandis</i> )	gills; mantle; whole organism	Cd defined in terms of $[Cd^{2+}]$ at sediment-water interface	Metallothionein increased 2.5-fold over the first 400 days in molluscs transferred from control lake to highly contaminated Lake Vaudray; increase in tissue [MT] correlated with increase in tissue Cd	Couillard et al. 1994

nmol/g wet wt in June 1985, this latter value being comparable to levels found in fish from control lakes (Deniseger et al. 1990).

As for the second possible use of MT, i.e. as a means of evaluating metal stress at the biochemical level, the responses of aquatic organisms to excess metal have proven rather more diverse than originally postulated; in some cases the initial binding is not to metallothionein but to the HMW fraction (Roesijadi 1982; Langston and Zhou 1987), in other cases both the HMW and the MT fractions increase in parallel (Roesijadi et al. 1982), and in still other examples the onset of toxic effects appears to be correlated not with the appearance of excess metals in the HMW fraction but rather with the detection and subsequent increase of metals in the very low molecular weight (VLMW) fraction (Sanders and Jenkins 1984). The use of the spillover model in a diagnostic sense is thus premature; any such application must await specific information on the metal detoxification mechanisms that are operative in the proposed biomonitor species (Engel and Roesijadi 1987; Roesijadi 1992).

#### 5.1.4 Summary

Metallothioneins offer a number of advantages as biomarkers for **exposure** to toxic metals in the (aquatic) environment (Stegeman et al. 1992) - e.g., presence of an extensive and increasing scientific information base; availability of sensitive analytical methods; concentration-dependent response to changes in ambient levels of (soft) metal cations. However, the biological functions of metallothionein are not fully understood and it is not yet possible to link changes in metallothionein status unambiguously to injury at the cellular or organismal level. In order to exploit metallothionein as a predictive indicator of **toxicity**, it will be necessary to expand our knowledge base relative to its normal physiological function. The basic biology/ physiology of the proposed biomonitor organism(s) must be known so that sources of variation other than metal exposure (e.g., growth and development; reproduction; food sources) can be taken into account.

## 5.2 Effects of metals at the population and community levels

### 5.2.1 Introduction

Much of our knowledge of the deleterious effects of metals on aquatic life has been provided by laboratory studies, which cannot be considered as representative of natural ecosystems. Despite this limitation, water quality criteria have frequently been based, in part, on data of this type. This approach has been criticized as being too simplistic and as providing inadequate protection for aquatic life (Kimball and Levin 1985; Cairns 1986, 1988; Schindler 1987). One alternative, also known as the holistic or ecosystem "health" approach, is based on an assessment of the status of the ecosystem using functional and/or structural properties of the system itself (Odum 1985; Schindler 1987; Munkittrick and Dixon 1989a, 1989b; Edwards et al. 1990; Rapport 1990; Ryder 1990). Ecosystem functions are processes (respiration, production, nutrient cycling), while ecosystem structure concerns the organisms present, organized into communities and populations.

Odum (1985) suggested that certain key features of ecosystem function and structure should be useful as indicators of contaminant stress and system degradation (Table 9). However, the normal variation of functional parameters can be quite extensive in unaffected systems and their relation to environmental factors such as temperature or climate is not well understood (Schindler 1987). Whole lake manipulation experiments have also shown that ecosystem functions can be accomplished via several pathways. This redundancy of these functions makes them poor predictors in systems undergoing chronic stress (Schindler et al. 1985; Schindler 1987). Schindler (1987) further suggested that structural components are the most sensitive indicator of a disturbance to an entire system. This would include communities - the biotic component of an ecosystem - and populations, the members of a species that occupy the same habitat and can possibly interbreed.

This section will focus on the structural components of ecosystems - the effects of metals on fish populations and on algal, zooplankton, benthic invertebrate and fish communities. Two points should be emphasized here. The first is the artificial separation of ecosystem components which are of course interrelated. This treatment reflects the focus of many of the studies cited, few of which deal with organisms outside the authors' area of specialization. However, the interactions of fish, zooplankton and phytoplankton are important in determining the productivity of aquatic ecosystems (McQueen et al. 1986; Northcote 1988; Carpenter 1991; Mills and Forney 1991; Crowder et al. 1991). According to the trophic cascade theory, the presence of predaceous fish influences the stability and productivity of the system; the effects of predators are "cascaded" through lower trophic levels such as planktivores and herbivores (Carpenter et al. 1985; Carpenter and Kitchell 1988; Northcote 1988). It is probable that these interactions would also be important in the responses of a single trophic level to contaminants. For example, both white sucker (*Catostomus commersoni*) and lake trout (*Salvelinus namaycush*) populations in Lake 223, an experimentally acidified lake, showed unexpected increases during acidification (Mills et al. 1987). It was suspected that the formation of algal mats provided young white sucker with increased protection from predation; these juvenile white sucker also possibly supplemented the diet of the lake trout (Mills et al. 1987). These type of interactions should not be ignored when considering the effect of contaminants on separate communities or populations.

A second point deals with the relative importance of structural and functional measurements. While structural measures are often considered as more sensitive than functional ones, the latter can be considered as better indicators of the ecological consequences of stress (Crosseley and La Point 1984). Such consequences can include the effects of structural changes on community metabolism and the capacity of the system to deal with contaminants (Crosseley and La Point 1984).

Table 9: Trends expected in stressed ecosystems (after Odum 1985).

Energetics:

1. Community respiration increases
2. Production/Biomass becomes unbalanced
3. Respiration/Biomass ratios increase
4. Importance of imported energy increases
5. Exported or unused primary production increases

Nutrient cycling:

6. Nutrient turnover increases
7. Horizontal transport increases and vertical cycling decreases
8. Nutrient loss increases

Community Structure:

9. Proportion of *r*-strategists increases\*
10. Size of organisms decrease
11. Lifespans decrease
12. Food chains shorten, disappearance of top predators
13. Species diversity decreases and dominance increases (if original diversity is low, the reverse may occur)

General system-level trends:

14. Ecosystem becomes more open, input and output become more important as internal cycling is reduced
15. Succession reverts to earlier stages
16. Efficiency of resource use decreases
17. Parasitism increases, mutualism decreases
18. Functional properties, such as community metabolism, are more robust than are species composition and other structural properties

\* see text (p. 40) for definitions of *r* and *k*.

A community can be characterized by measurements of the total abundance, density or biomass of all individuals, the total number of species (the species richness), the relative abundance of species (evenness), or by the most common or dominant taxa (family, genus or species). Species usually found together, or associations, can be used to typify a habitat. The diversity of a community can also be expressed as an index, which measures the uncertainty in predicting the species of the next individual sampled (Washington 1984; Fausch et al. 1990). The effects of pollution are often most evident as changes either in the density or abundance of individuals, or in community composition and dominance, as sensitive species are replaced by tolerant ones (Clements 1991; Luoma and Carter 1991; Hare 1992). Communities, in particular those comprising periphyton and benthic insects, have been quite commonly used to evaluate the impact of metals.

The characteristics of a population include abundance, productivity and persistence (Goodyear 1983). Fish populations have often been used as bioindicators in aquatic systems, in part because of their economic and aesthetic value to society. For example, the lake trout population in Lake Ontario has recently been proposed as an indicator of the "health" of the entire system (Edwards et al. 1990; Ryder 1990). Munkittrick and Dixon (1989a, 1989b) have proposed an approach using white sucker (*Catostomus commersoni*) for the evaluation of stress associated with low levels of contaminants. In their framework, the characteristics of a population suffering from contaminant stress should fit one of five patterns or response types, which are indicative of effects on certain life stages. The approach includes measurements of several parameters that are essential in order to assess the average health, reproductive effort, growth, abundance and recruitment of a population (Table 10). Other factors that may usefully be measured include effects on diet and on the use of habitat (Goodyear 1983).

## **5.2.2 The effects of metal stress on communities**

### **5.2.2.1 Algal communities**

The composition of the algal community in freshwaters has often been used as an indicator of water quality, especially in streams and rivers (Crosseley and La Point 1984; Rai et al. 1985; Clements 1991). Changes in this community - the number of species present, its diversity or overall composition, or its dominant species - are often observed in the presence of contaminants (Stokes 1983; Whitton 1984).

Buttle Lake has been the site of numerous studies examining the impacts of mine wastes on lake communities. In 1966, a Cu-Zn mine began operations in the vicinity of the lake, which is situated on Vancouver Island, British Columbia. Metal concentrations in the lake increased until abatement measures were instigated in 1980, and metal levels began to decline in 1984. The species composition changed as metal tolerant species became dominant, replacing those species usually found in oligotrophic B.C. lakes (Austin et al. 1985; Deniseger et al. 1990). The total number of species (species richness) and the community diversity also decreased. With declining metal levels, tolerant species gradually were replaced as metal-sensitive genera and species returned to dominance (Austin et al. 1985; Deniseger et al. 1990). In particular, one previously dominant species (*Rhizosolenia*

*eiseneri*) disappeared in 1983, recovered dominance in 1984, and virtually excluded all other algae from the lake in 1985.

It has been suggested that the dominance of certain algal taxa can be used as a diagnostic tool for evaluating the degree of metal contamination (Foster 1982; Crosseley and La Point 1984; Clements 1991). While the results from Buttle Lake appear to support this contention, other factors contribute to dominance of one species or another in polluted waterbodies. Prior to 1984, *R. eiseneri* had been considered as a "metal-sensitive" species, yet its recovery and predominance in later years occurred at metal concentrations that had previously caused its decline. The recovery was also associated with an increased availability of nitrogen in the lake, the source of which was N-rich mine waste. This increased supply, coupled with this organism's high requirement for N, possibly allowed it to dominate the lake during recovery (Deniseger et al. 1990).

Normally sensitive taxa have also been found to develop metal tolerance and thus reliance upon the presence of a certain genus or species as indicative of metal contamination is not recommended (Foster 1982; Stokes 1983, Whitton 1984).

Dominance changes associated with metal contamination may also be related to increased or reduced grazing pressure (Langston 1990). Yan (1979) and Yan and Strus (1980) studied acidic metal-contaminated lakes in Ontario. Algal communities in stressed lakes had reduced species richness, decreased biomass and were of a different composition when compared to reference lakes. The effects were most severe in acid lakes if metals were also present. However, the dominant zooplankton in these lakes were small organisms, such as *Bosmina longirostris*, which also feed on small sized algae (Yan and Strus 1980). By reducing the numbers of small algae, these organisms may have contributed to the dominance of larger species in these lakes. The interactions of phytoplankton and zooplankton are important factors to consider when determining algal dominance.

Changes in the composition of algal communities, generally reductions in species richness, have been correlated with the degree of pollution in metal-contaminated streams (Foster 1982; Crosseley and La Point 1984; Clements 1991). Algal community composition was also considered as the most sensitive indicator of the state of an experimentally acidified lake (Schindler *et al.* 1985; Schindler 1987). Changes in dominant algal species may also be detected using paleoecological techniques; these changes can be correlated with suspected changes in water quality, such as acidification and elevated levels of metals (Dixit et al. 1988a; 1988b). However, this approach is limited to algae that can be fossilized (diatoms, chrysophytes) and is not specific for metal contaminants.



Table 10: Population parameters for the assessment of low-level contaminant stress in fish populations (Munkittrick and Dixon, 1989)

Type of response, life stage affected	mean age	age distribution	growth rate	condition factor <sup>1</sup>	age at maturation	R.L.S. <sup>2</sup>	fecundity	egg size	CPUE <sup>3</sup>	population size
1 direct on adults	-	-	+	+	-	0, -	-, +	-, +	-	-
2 direct on eggs, larvae	-	+	0, +	0	0	+	0	0	-	-
3 direct on juveniles (multiple stress)	+	+	-	-	+	0	-	-, 0	-	-
4 indirect, above indicator organism (predator)	0	0	-	-	+	-	-	-	+	+
5 indirect, below indicator organism (prey)	0	0	-	0	+	-	-	-	-, 0	-, 0

1. condition factor: =  $100 (\text{wt}/(\text{length}^3))$ .

2. reproductive life span = mean age - (age to maturation).

3. catch per unit effort.

#### 5.2.2.2 Crustacean zooplankton

Few studies have examined the impacts of metals on lake communities of crustacean zooplankton, despite their importance as a prey item for fish. In Buttle Lake, calanoid copepods and cladocerans decreased in numbers until 1984, at which point almost the entire community had disappeared (Deniseger *et al.* 1990). The abundance of individuals and the diversity of the community began to recover after this date and in 1986 the community diversity and composition resembled that observed in 1966-67. Similar results have been reported for lakes both during acidification and after recovery. In acid lakes, decreases in diversity, total biomass and changes in dominance among crustacean zooplankton have also been observed (Sprules 1975; Yan and Strus 1980; Confer *et al.* 1983; Schindler *et al.* 1985). Keller and Yan (1991) reported that increases in zooplankton community species richness and the return of acid intolerant species occurred in lakes recovering from acid and metal contamination.

In the Experimental Lakes Area, the effects of additions of Cd have been studied. Lawrence and Holoka (1987) added Cd to lake enclosures, as the pH of the lake was gradually lowered over several years, and observed the effects on the zooplankton community. There were reductions in community biomass and in the number of species at low pH, yet Cd appeared to mitigate some of the effects of acid pH. At pH 5.6, the effects on biomass were less severe in the presence of Cd, while the metal did have effects on the species composition. Low levels of Cd (1.78 nM) have also been added to the epilimnion of Lake 382 over a period of 4 years (Malley *et al.* 1990). While there was no clear evidence of community-level effects, the authors suggested that zooplankters avoided the epilimnion when Cd concentrations were elevated.

#### 5.2.2.3 Benthic invertebrates

The benthic community is in intimate contact with both water and sediment phases and is also a source of food for birds and fish. The impact of metals on benthic communities has been extensively studied in streams polluted by mine tailing residues, acidic mine drainage or by experimental additions of metals (Clements 1991; Hare 1992). The existence of gradients in streams allows comparisons among impacted sites, as well as with upstream and downstream reference sites. Attempts have been made to correlate the biological changes observed with distance from the source or with the degree of contamination (Clements 1991). The effects noted have included reductions in the number of taxa (taxonomic richness: Clements *et al.* 1988, 1992), changes in the proportion of tolerant vs. sensitive taxa (Winner *et al.* 1980; Sheehan and Winner 1984; Clements *et al.* 1992), the losses of selected representative taxa (Moore *et al.* 1991) and reductions in the density (Chadwick and Canton 1984; Sheehan and Winner 1984; Chadwick *et al.* 1986) and abundance of individuals (Clements *et al.* 1988, 1992).

The complexity of a community is frequently expressed as a diversity index, a measurement of the species richness weighted with the abundance of individuals per species (Washington 1984). However, such traditional ecological measurements are not always useful in detecting the effects of pollution. In metal-polluted streams, benthic community diversity is not generally indicative of the degree of contamination (Winner *et al.* 1980; Chadwick and

Canton 1984; Chadwick et al. 1985). Chadwick and Canton (1984) studied sites along Silver Bow Creek, a Montana stream polluted by mine waste. At contaminated sites, organism density was reduced without corresponding decreases in the number of species. These effects were not reflected in the diversity indices they tested. Existing diversity indices, such as the Shannon-Weiner Index, do not take these changes into account. Many diversity indices are strongly influenced by sample size (Winner et al. 1980; Chadwick et al. 1985) and tend to over-represent species with few individuals (Sheehan 1984). Measurements of diversity are thus of limited usefulness for the detection of community changes, apart from situations of severe impact (Clements 1991).

In cases of severe metal pollution, however, communities do tend to resemble one another and the use of similarity indices has been useful in identifying these impacted sites (Winner et al. 1980; Clements et al. 1988, 1992). From their work on the Clinch River (Virginia), Clements et al. (1992) developed an index of common sensitivity (ICS) to differentiate reference and impacted sites. The ICS is a measure of the similarities in community composition, and in the number of individuals and taxa present, based on 13 dominant benthic taxa. It was applied to both the Clinch River and experimental stream sites with some success, and could be used on other polluted rivers (Clements et al. 1992).

Proportions of tolerant and sensitive taxa at different sites have also been used as indices of the degree of metal stress in streams (Winner et al. 1980; Clements et al. 1988, 1992; Moore et al. 1991). Winner et al. (1980) and Clements et al. (1988) have suggested a simple scale wherein severe pollution is indicated by the dominance of chironomids (*Chironomidae*), moderate pollution by caddisflies (*Trichoptera*) and chironomids, and the least pollution by caddisflies and mayflies (*Ephemeroptera*). The *Chironomidae*, and in some cases the *Oligochaetae* (Chapman et al. 1985), are apparently tolerant of severe pollution, while the *Ephemeroptera* are considered as extremely sensitive (Winner et al. 1980, Clements et al. 1988, 1992; Moore et al. 1991).

However, the classification of entire taxa as tolerant or sensitive may ignore important differences that exist within certain taxa. Hare (1992) points out that sensitivity within orders of insects varies; for example, certain Ephemeropteran genera appear more tolerant of metals in certain habitats than in others. Clements et al. (1992) have determined that at least one chironomid tribe - the *Tanytarsini* - is as sensitive to metals as are mayflies. Chapman et al. (1985) noted that the sensitivity to pollution differs among the oligochaetes. The interpretation of results, and the classification of taxa as tolerant or sensitive, is often dependent on the level of taxonomic resolution (Winner et al. 1980). Thus the classification of habitats as polluted or not by the presence or absence of orders may be inaccurate.

In addition, the different ecological roles of the organisms are ignored when species, genera or orders are pooled and treated as one unit (Hare 1992). Trophic interactions may be simplified in impacted habitats (Sheehan 1984) yet relatively few studies have attempted to account for the ecological niche of either sensitive or tolerant organisms. Moore et al. (1991), in studies of a mountain river affected by acidic mine drainage (the watershed of the Blackfoot River, Montana), found that predaceous species were more sensitive than detritus

feeders to metal pollution. In contrast, other studies suggest that predators are less affected by metal exposure than other functional groups (Selby et al. 1985; Leland et al. 1989).

In many stream studies, temporal and spatial variability has complicated the analysis and interpretation of results (Sheehan and Winner 1984; Clements et al. 1992). Coefficients of variation (Sheehan and Winner 1984), as well as more sophisticated statistical techniques such as cluster (Chadwick et al. 1986) or multivariate analyses (Clements et al. 1992), have been used to compare sites and to detect trends despite significant variation. The importance of seasonal variation was emphasized by Hare and Campbell (1992), who found that there was significant seasonal variation in accumulated metals in several insect taxa in lakes. Organisms from the same sites were compared over an annual cycle and the accumulation of cadmium was found to vary seasonally, yet not all metals responded in a similar manner.

Measurements of functional and population parameters in impacted communities have also been rare. Sheehan and Knight (1985) combined structural and functional parameters in a "multilevel" approach, studying sites along Little Grizzly Creek. They measured the taxonomic composition (abundance of genera, species richness, and the dominant taxa), in addition to the secondary production and production:biomass ratio, of the chironomid community. Recruitment of early instar larvae was decreased during the fall, though it was difficult to trap all of these smaller organisms because of downstream drift. However, they postulated that energy was lost from the system as food (algae, periphyton) was being exported.

The impact of metals on benthic communities in lakes has received proportionally less attention (Hare 1992). Moore (1979) considered that density of organisms was the best indicator of pollution in Great Slave Lake (NWT), a sub-arctic lake affected by mining effluent. Species composition and diversity were similar in heavily contaminated sites and in relatively clean ones, while density increased with distance from the source of effluent. Moore (1979) also concluded that diversity indices may not be applicable in sub-arctic environments, due to the low species counts.

Munkittrick et al. (1991) also found that density, as well as the numbers of taxa, of benthic invertebrates were reduced in a metal-contaminated lake, in comparison with a reference lake. Lake Manitouwadge, part of a chain of lakes in Northern Ontario, has been severely contaminated by copper and zinc derived from mine tailings. *Chironomidae* and *Copepoda* were dominant taxa in both lakes, yet the order of dominance was reversed in the contaminated lake. Other invertebrates (*Amphipoda*, *Ephemeroptera*, *Gastropoda*, non-dipteran insects) commonly found in the region were totally absent in Lake Manitouwadge. Effects observed in the indigenous white sucker (*Catostomus commersoni*) were linked to the disappearance of these taxa (Munkittrick and Dixon 1988a; Munkittrick et al. 1991).

#### 5.2.2.4 Fish communities

Most studies involving fish communities have involved listing the species present (community composition), measuring the species richness (the number of species) or using the associations of species, which are considered to prefer certain habitats, as indicative of water quality (Karr 1981; Fausch et al. 1990). As with other communities, the presence of tolerant, or the absence of sensitive, species is not necessarily indicative of pollution (Fausch et al. 1990). The use of diversity indices either to predict or to measure the degree of contamination in rivers and streams has also met with mixed results, because fish species diversity is known to vary substantially with season and with year in these habitats (Fausch et al. 1990).

Population density and total biomass are frequently used measurements of the state of stream communities. In Welsh streams affected by metal and acid runoff, Turnpenny et al. (1987) found that the presence of fish, the population density and the total biomass were affected by low pH. In certain streams, the presence of metals (Al, Zn, Mn) accounted for the effects observed. In acidified Nova Scotia rivers, fish communities had lower density, lower biomass and shifts in species composition, when compared to neutral pH systems (Lacroix 1987).

Somers and Harvey (1984) sampled fish communities in lakes receiving atmospheric inputs of acid and metals from a smelter in the vicinity of Wawa, Ontario. Part of the study involved tabulating population losses. Many lakes were fishless and historical data suggested that previously existing populations, in particular the top predator species brook trout (*Salvelinus fontinalis*) and the lake trout (*Salvelinus namaycush*) had become extinct. Associations of species, especially those species considered as sensitive, were also examined. In many lakes, the commonly co-occurring species of Northern pike (*Esox lucius*), walleye (*Stizostedion vitreum vitreum*), and yellow perch (*Perca flavescens*), appeared most affected. The frequency of occurrence (% of lakes with fish) and the species richness increased with distance from the source of pollution. Associations of species were also used as indicative of metal and acid stress. Lakes closest to the smelter were also characterized by "unusual" species associations and by communities dominated by cyprinids. Many studies of the effects of acidic precipitation (Wright and Snekvik 1978; Harvey 1982; Minns et al. 1990) have used similar approaches and have shown similar effects on fish communities in lakes (absence of fish, reduced species richness, decreased frequency of occurrence).

### **5.2.3 Effects of Metals on Fish Populations**

Recent studies examining the effects of metals on fish populations have been concerned with either the impacts of atmospheric deposition due to smelter operations, or the influence of mine tailings. The source of the metal is important when fish population characteristics are measured. Hamell Lake, Saskatchewan, has been contaminated by metals (mainly Zn and Cd) originating from a nearby smelter (Van Loon and Beamish 1977). McFarlane and Franzin (1978) found that the native white sucker population grew faster, had higher

fecundity (number of eggs per female), had fewer adults, and was on average younger than the reference population in Thompson Lake. They also found that the females matured earlier, but reproduced less successfully. They concluded that the population attempted to compensate for this reduced spawning success by increasing their reproductive effort (McFarlane and Franzin 1978). Populations that attempt to maximize their rate of reproduction are following a strategy of " $r$ " selection, so-called because  $r$  represents the intrinsic natural rate of increase of the population.

Lake Manitouwadge, discussed previously, had similar total metal concentrations (Zn and Cu) in the water column, and a similar water chemistry (pH and hardness) to Hamell Lake (McFarlane and Franzin 1978; Munkittrick and Dixon 1988a; 1989b). However, effects on the white sucker population were considerably different. In comparison with the reference population in Loken Lake, the Lake Manitouwadge population exhibited smaller egg size, reduced larval growth rates and survival (Munkittrick and Dixon 1989c) and had reductions in growth rate and in fecundity, yet there were no differences in either age of maturation or in condition (Munkittrick and Dixon 1988a).

Due to the fact that the size of the population has not changed despite the reduced larval survival, Munkittrick and Dixon suggested that the carrying capacity ( $k$ , the number of individuals a habitat can support) of the habitat had been reduced. In later studies (Munkittrick and Dixon 1991; see above), evidence was presented to support their hypothesis of a niche shift by this population (the replacement of preferred prey species by less nutritious ones in the diet). The population would thus be forced into a new equilibrium at a lower  $k$ .

These two studies show the importance of population strategies ( $r$  vs.  $k$ ) in adapting to stress, a factor that should be considered when measuring population characteristics. In addition, the source of contamination was evidently important for the effects observed in the two white sucker populations. In lakes contaminated by atmospheric deposition, such as Hamell lake, the primary effects of increasing metal levels may be on the young larval stages. In the case of mine tailings (eg Lake Manitouwadge), benthic invertebrates, the favored prey of white sucker, may be the first organisms affected.

Of course, the biology of the species under study, as well as its habitat, would play a role in the importance of niche shift in a population. Turnpenny et al. (1987) studied the diet of brown trout (*Salmo trutta*) inhabiting low pH and metal polluted streams in Wales. They classified organisms found in the gut, as well as comparing stomach fullness. Comparisons with populations in neutral pH streams revealed that there were no differences, despite the reductions in prey fauna in the low pH-metal stressed habitats. The latter trout populations appeared to supplement their diet from terrestrial sources.

Another important aspect arising from studies of natural populations is their tolerance of waterborne metal concentrations that are apparently toxic. Van Loon and Beamish (1977) proposed that organisms in lakes near a smelter, including those in Hamell Lake, were more tolerant of high zinc concentrations than what would be expected from laboratory toxicity data. On the basis of data obtained in 1981, Klaverkamp et al. (1990) reported that Hamell lake white suckers were much more resistant to lethal Cd levels than were fish from

relatively non-contaminated Thompson Lake. White sucker larvae, originating from Lake Manitouwadge and raised from the egg stage in clean laboratory water, were more tolerant of metals (Cu, Zn) during toxicity tests than were similarly treated larvae from Lake Loken, the reference lake (Munkittrick and Dixon 1988b; 1989c). The concentrations of metals present in Buttle Lake caused mortality to rainbow trout (*Oncorhynchus mykiss*) under laboratory conditions (Roch and McCarter 1984a). In addition, many of the trout prey species in Buttle Lake (*i.e.* crustacean zooplankton) suffered almost total extinction at one point in time (Deniseger et al. 1990), yet trout populations continued to exist, apparently successfully (Roch et al. 1982; Deniseger et al. 1990). The maintenance of populations at these elevated metal levels suggested the existence of tolerance mechanisms, such as the production of metallothioneins (Hamell Lake: Klaverkamp et al. 1990; Buttle Lake: Roch et al. 1982; Roch and McCarter 1984b). However, the fact that these mechanisms have been shown to exist in natural populations does not imply that these populations are not at risk. As of 1986, the Hamell Lake white sucker population appeared close to extinction (Klaverkamp et al. 1990).

#### 5.2.4 Summary

Recent studies suggest that chronic exposure to low levels of contaminants eventually results in severe effects on populations (Munkittrick and Dixon 1989) and communities (Schindler 1987) in aquatic environments. Thus, community and population data are essential for the prediction and monitoring of these effects, in combination with appropriate toxicity tests (Schindler 1987) and the use of biochemical and physiological indicators (Munkittrick and Dixon 1989a).

Difficulties may be encountered in relating contaminants to observed effects due to natural variation or to unrelated environmental changes. The effects of natural population cycles, climatic effects and other environmental factors not related to contamination, must be accounted for if the impacts of contaminants on populations and communities are to be properly assessed (Munkittrick and Dixon 1989a; Schindler 1987; Schindler et al. 1985). Lessons may be learned from the lake manipulation studies carried out in the Experimental Lakes Area in Ontario. The results of the acidification studies in particular have emphasized the importance of representative reference sites and the benefits of an extensive chemical and biological data base. In addition, population and community parameters were monitored over several years, which allowed the changes observed to be linked to the experimental manipulation (Schindler et al., 1985; Schindler 1987; Mills et al. 1987; Mohr et al. 1990).

Community level studies, which encompass several trophic levels, can be essential in interpreting the response of one population to a stress (metal mine waste: Munkittrick and Dixon 1988a; acidification: Mills et al. 1987). This approach avoids the possible exclusion of the most sensitive components of a community, which might occur if attention is concentrated on a single species. From an ecological perspective, the feedback mechanisms that exist among trophic levels and between competitors are important in determining dominance in an algal community (Yan and Strus 1980) or survival of young fish (Mills et al. 1987).

Certain community indices have been shown to be more useful than others in detecting the impacts of contaminants such as metals or acid. The most useful appear to be species composition (Schindler et al. 1985; Schindler 1987; Deniseger et al. 1990) and species richness (Austin et al. 1985; Deniseger et al. 1990). In the limited number of lake studies available, the density of benthic organisms has proven useful (Moore 1979; Munkittrick et al. 1991), though this may not be the case in less severely contaminated habitats. Improved methods for the taxonomic differentiation of benthic animals are needed both to improve the relationship of community composition with the degree of metal stress and to place these effects in an ecological context (Hare 1992).

Fish populations are not necessarily the most sensitive predictors of the future state of a system (Schindler et al. 1985). However, there are certain advantages to the use of fish populations, notably the relative facility of collecting the data necessary for such studies (age of individuals, reproductive status, growth, condition) and the existence of historical records for comparison. In the appropriate context, fish population assessments are an integral component for the evaluation of contaminant impacts (Munkittrick and Dixon 1989a; 1989b). Furthermore, population data are essential to relate chronic toxicity, often observed in individuals in the field, to effects at the level of the population. In their studies of metallothionein in European perch (*Perca fluviatilis*), Hogstrand et al. (1991) also noted differences in sex ratio and body weight between populations in their contaminated and reference sites. However, there was insufficient population data to evaluate whether these differences were associated with metal exposure.

Finally, the importance of metal bioavailability should be considered. In many of these studies, the chemistry of the exposure milieu (sediments, water column) was insufficiently defined, thus precluding reasonable speculations about the amount of metal that was actually bioavailable. Comparisons between lakes, or even within the same lake over time, are hazardous without accounting for the fraction of total metal that causes the observed biological effects.



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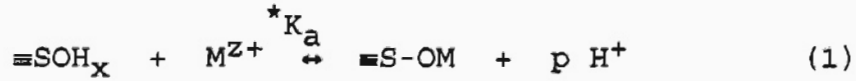
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**7. APPENDIX 1 - DERIVATION OF THE SURFACE COMPLEXATION  
MODEL FOR METAL SORPTION ON SURFICIAL SEDIMENTS**

It is assumed that: (i) the two main components of oxic sediments that sorb metal **M** are iron oxyhydroxides (Fe-ox) and sediment organic matter; (ii) these components have surface sites for sorption that can be treated as surface ligands; and (iii) surface complexation concepts that have been developed for simple, well-defined systems in the laboratory can be applied to more complex sediment components. According to surface complexation theory, sorption of **M** can be expressed in a simplified manner as:



$$*K_a = \frac{\{\equiv\text{S-OM}\} [\text{H}^+]^p}{\{\equiv\text{SOH}_x\} [\text{M}^{z+}]} \quad (2)$$

where charges on the solid species are omitted for simplicity; S = sorbent (either amorphous Fe-ox or sediment organic matter);  $*K_a$  is an apparent overall equilibrium constant; "=" refers to sorption sites and p = the average number of protons released per metal ion sorbed.

At low sorption densities, i.e. when the concentration of occupied sorption sites is low relative to the free site concentration, the condition  $\{\equiv\text{S-OH}_x\} \approx \{\equiv\text{S-O}\}_T$  should apply, where  $\{\equiv\text{S-O}\}_T$  is the total concentration of sorption sites. This term can in turn be related to the analytical concentration of the sorbents,

$$\{\equiv\text{S-O}\}_T = N_S \cdot \{\text{S-OH}\} \quad (3)$$

where  $\{\text{S-OH}\}$  = the analytical concentration of the sediment component acting as sorbent, e.g.  $\{\text{Fe-ox}\}$ ;  $N_S$  = the number of moles of sorption sites per mole S-OH. If it is further assumed that each sorbed metal ion occupies a single site, then

$$\{\equiv\text{S-OM}\} = \{\text{S-OM}\} \quad (4)$$

Combining equations (2), (3) and (4) yields

$$[M^{Z+}] = \frac{\{S-OM\}}{\{S-OH\}} \cdot \frac{[H^+]^p}{N_S \cdot K_a} = \frac{\{Fe-OM\}}{\{Fe-ox\}} \cdot \frac{[H^+]^x}{N_{Fe} \cdot K_a} \quad (5)$$

which shows how one might calculate the free-ion concentration,  $[M^{Z+}]$ , provided that the value of  $N_{Fe} \cdot K_a$  is known. Note that  $\{ \}$  and  $[ \ ]$  refer to concentrations of solids and dissolved species respectively.

To estimate  $N_{Fe} \cdot K_a$ , equation (5) can be rearranged as follows

$$K_{Fe-M} = \frac{N_{Fe} \cdot K_a}{[H^+]^x} = \frac{\{Fe-OM\}}{\{Fe-ox\} [M^{Z+}]} \quad (6)$$

where  $K_{Fe-M}$  is an apparent equilibrium constant which is a function of pH. Values of  $\{Fe-OM\}$  and  $\{Fe-ox\}$ , needed to estimate  $K_{Fe-M}$ , can be obtained by selective extraction of the surficial sediments; values of  $[M^{Z+}]$  can be obtained by *in situ* dialysis (porewater peepers) - see Tessier (1992) and Tessier et al. (1993).

Equation (6) can then be linearized

$$\log K_{Fe-M} = x \text{ pH} + \log (N_{Fe} \cdot K_a) \quad (7)$$

and a plot of  $\log K_{Fe-M}$  vs. pH should yield a slope of  $x$  and a Y-intercept of  $\log (N_{Fe} \cdot K_a)$ . Values of " $x$ " and " $\log (N_{Fe} \cdot K_a)$ " obtained in this manner are summarized in Table 2 (p.5).