How to Assess Potential Biological Effects of Subaqueous Disposal of Mine Tailings – Literature Review and Recommended Tools and Methodologies

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Literature Review and Recommended Tools and Methodologies

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Glossary

absorb	cross a biological membrane and enter a living organism
adsorb	bind to a surface (living or nonliving)
AE	assimilation efficiency (applies to diet-borne metals)
allochthonous particles	particles originating from the catchment or from atmospheric deposition
anaerobic or anoxic	devoid of oxygen
ARD	acid rock drainage; drainage from geologic materials with insufficient capacity to neutralize acidic products from oxidation of sulphide minerals or intermediary sulphur species, or from the dissolution of acidic minerals and amorphous materials produced by previous sulphide oxidation
autochthonous particles	particles generated within a water body, for example by algal photosynthesis, breakdown of aquatic vegetation, or in situ mineral precipitation
AVS	acid volatile sulphide
BAF	bioaccumulation factor ($[M]_{\text{animal}}/[M]_{\text{food}}$)
BAM	biologically available metal
BCF	bioconcentration factor ($[M]_{\text{plant or animal}}/[M]_{\text{water}}$)
benthic	relating to, or occurring at the bottom, or in the sediments, of a body of water
biotic ligand	metal-binding moiety or ligand that is an integral part of a living organism; often corresponds to a metal transporter or a metal-sensitive site, both present in an epithelial membrane and thus available for interaction with a waterborne metal
BIM	biologically inactive metal
bioaccumulation	accumulation (e.g., of a metal) within a living organism
bioirrigation	action of benthic invertebrates, irrigating their burrows in sediment
biomagnification	increase in metal concentrations along the food chain, from lower levels (detritus, algae) to higher consumers including zooplankton, benthic invertebrates and fish; using considered over three trophic levels
bioturbation	action of benthic invertebrates contributing to the physical mixing of sediment
BLM	biotic ligand model
CCME	Canadian Council of Ministers of the Environment
cDNA	complementary DNA (deoxyribonucleic acid)
contaminated sediment	sediments in which the metal levels are (well) above the local geological background concentration (such elevated concentrations may result from anthropogenic activities or from natural surficial mineralization)
DET	diffusion equilibration in thin films technique
DGT	diffusion gradient in thin films technique
diagenesis	physical and chemical changes occurring during the conversion of sediment to sedimentary rock
DO	dissolved oxygen
DOC	dissolved organic carbon
DOM	dissolved organic matter
DSR	dissimilatory sulphate reduction
DYM-BAM	dynamic multi-pathway bioaccumulation model
e-DNA	environmental DNA (deoxyribonucleic acid)

EC_x	effective concentration affecting 'x' percent of the test population
EEM	environmental effects monitoring
epithelial membrane	biological membrane that separates a living organism from its abiotic environment
EU	European Union
eutrophic	adjective, applies to nutrient-rich, biologically productive water bodies
EXAFS	extended x-ray absorption fine structure
FIAM	free-ion activity model
fyke net	bag net for catching fish
GI	gastrointestinal
granulometry	measurement of the size distribution of grains in a solid sample (sediment, soil, tailings)
hard cation	cation that is small, with a high charge state and low polarizability (e.g., Na, K, Ca, Mg, Al, Fe)
HC_x	hazard concentration, at which 'x' percent of the species in a species sensitivity distribution (SSD) exhibit an effect
homeostatic control	ability of living organisms to maintain the concentrations of certain key solutes in a narrow range (e.g., by increasing uptake when the intracellular concentrations drop too low and by increasing efflux when the intracellular concentrations rise above a certain threshold)
HPLC	high performance liquid chromatography
HDP	heat-denatured proteins
HSP	heat-stable proteins
IC_x	inhibitory concentration affecting 'x' percent of the test population
IR	ingestion rate (applies to diet-borne metals)
limiting factor	nutrient, growth factor or property of the environment that restricts the growth of an organism
littoral	near-shore aquatic environment (river, lake) where light can penetrate to the benthic boundary, sometimes supporting growth of aquatic plants
LOEC	lowest observed effect concentration
macrophyte	aquatic plant that grows in or near water and is either emergent, submerged, or floating
MATC	maximum allowable toxicant concentration
MEND	Mine Environment Neutral Drainage programme
metallothionein	cysteine-rich proteins involved in maintaining sufficient intracellular supplies of some essential metals (Cu, Zn) and in detoxifying excess intracellular metals (including non-essential metals, e.g., Cd)
microarray	collection of microscopic DNA spots attached to a solid surface; used to measure the expression levels of large numbers of genes simultaneously
ML/ARD	metal leaching/acid rock drainage
ML_n	metal complex involving the cation M and ligand L; the n refers to the number of ligands bound to the metal cation
MMME	metal mixtures modelling exercise
mRNA	messenger RNA (ribonucleic acid)
M^{z+}	free metal cation, with a positive charge of '+z'
NGS	next generation sequencing
NOEC	no observable effect concentration
oxidation	*loss* of electrons or an *increase* in oxidation state by a molecule atom or ion

periphyton	complex biofilms of algae, cyanobacteria, heterotrophic microbes and detritus that attach to submerged surfaces
plankton	micro-organisms that live in the water column and are generally incapable of swimming against a current (phytoplankton refers to algae or plants, zooplankton to animals)
PNEC	predicted no-effect concentration
reclamation	restoration of productivity and usefulness, although the uses may change from those previous to mining
redox	abbreviation for reduction-oxidation; a redox reaction is one in which the oxidation states of the atoms involved are changed
reduction	*gain* of electrons or a *decrease* in oxidation state by a molecule atom or ion
riparian	adjective, referring to the banks of a natural water body
SAD	subaqueous disposal
SEM	simultaneously extracted metal
SEC	size exclusion chromatography
SETAC	Society for Environmental Toxicology and Chemistry
SRB	sulphate-reducing bacteria
SSD	species sensitivity distribution
TAM	trophically available metal
tortuosity	term describing the convoluted path dissolved ions must follow to circumvent sediment particles along a diffusive flow path
transcriptome	set of all RNA molecules in one cell or a population of cells (usually just mRNA), reflecting the genes that are being actively expressed; can be used as an indication of the response of an organism to an external stress
TTF	trophic transfer factor
VISUAL MINTEQ	a chemical equilibrium model
WHAM	Windermere Humic Aqueous Model
XANES	x-ray absorption near edge structure
XAS	x-ray absorption spectroscopy
XRF	x-ray fluorescence

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Executive summary

Context

Subaqueous disposal (SAD) or flooding of sulphide-rich tailings in constructed facilities is one of a number of mitigation methods used by mines. The primary mitigation mechanism resulting from subaqueous disposal is limitation of oxygen ingress into water filled pores, which greatly reduces sulphide oxidation, minimizes metal leaching and prevents the development of acidic drainage.

To date, most studies and monitoring activities related to closed SAD facilities have focused on the initial physical and geochemical performance of the facilities and the resulting chemistry of the surface water. Longer-term aspects, such as the progressive addition of natural organic matter by sedimentation on top of the tailings and the overall biological performance of such facilities, are not as well understood. In particular, a major gap in understanding relates to the biological colonization of such facilities, the health of biological communities that are established, and the influence of those communities on water and sediment geochemistry.

Literature reviews in this general area were commissioned by the Mine Environment Neutral Drainage (MEND) programme in 1993 and 2009. These reviews focused on possible means of evaluating the potential biological effects of subaqueous disposal of mine tailings, with an emphasis on the fauna and flora that had colonized the tailings impoundment. Building on these earlier reports the present study was designed (i) to provide updated information on potential diagenetic changes in submerged tailings and on biogeochemical interactions between submerged tailings and overlying aquatic communities, and (ii) based on the information obtained in part (i), to provide guidance regarding recommended tools and methodologies that could be used to predict and/or monitor the biological effects of submerged tailings.

Contaminants addressed in the present preview fall into two categories: (i) the data-rich cationic trace elements that were covered in the previous reviews (Cd, Cu, Pb, Ni and Zn) and (ii) trace elements forming oxyanions and neutral polyhydroxy species that were not dealt with in the previous reports (As, Mo, Sb, Se). Other elements, such as Al, Fe and Mn, have been considered in the context of the diagenetic changes that occur in sediments and tailings after their deposition in a subaqueous tailings facility.

Content

The present report includes an Introduction (Section 1), Literature Review (Sections 2 to 7) and a set of Recommendations (Section 8). The Literature Review first considers how waterborne and diet-borne metals interact with living organisms (Sections 2 and 3), and then integrates these two uptake vectors and considers metal bioavailability in sediments and in submerged tailings (Section 4). This latter section also includes examples of various biomonitoring approaches that can be used to assess metal bioavailability in a SAD facility. The Literature Review also addresses the relative sensitivity of different aquatic organisms to the metals of concern (Section 5) and offers an overview of the environmental chemistry of various oxyanions and their bioavailability (Section 6). The final part of the Literature Review deals with data generated from the assessment of SAD facilities in Canada and elsewhere, found in company and government reports and conference proceedings (Section 7). The Recommendations section draws upon all this information and provides guidance regarding recommended tools and methodologies that could be used to assess the biological effects of submerged tailings.

Results

Tools

In the earlier MEND reviews, various approaches or tools were presented, notably: (i) the Biotic Ligand Model (BLM) to evaluate the bioavailability of metals present in the overlying water or in the tailings pore water; (ii) the Acid Volatile Sulphide – Simultaneously Extracted Metals (AVS–SEM) model to evaluate the potential toxicity of the metals present in submerged, aged and anoxic tailings; (iii) sorptive equilibrium models to calculate the free cation concentrations in the oxic tailings pore waters.

- The BLM was already reasonably mature by 2008 and the approach has undergone considerable improvement since that time, notably in its ability to handle chronic toxicity (refinements to the metal speciation component of the model) and in its application to metal mixtures. The review in the MEND (2009) report is still an accurate and useful summary, particularly for the well-studied metals (Cd, Cu, Ni, Pb, Zn). To evaluate the bioavailability of these metals, in dissolved form (i.e., in the water that overlies submerged tailings and in the pore water residing in the tailings interstices), the BLM approach remains the recommended tool to use.
- The AVS–SEM model and the sorptive equilibrium model are both designed to yield estimates of the free metal cation concentration in sediment or tailings pore water, this being recognized as the best way to evaluate the bioavailability of a sediment-associated metal. The former applies to anoxic conditions and assumes that pore-water metal concentrations are controlled by precipitation-dissolution reactions with reactive amorphous sulphides (AVS), whereas the latter was calibrated for oxic conditions and assigns control of the pore-water metal concentrations to sorption reactions on such sorbants as Fe-, Mn-oxyhydroxides or sediment organic matter. In the 20+ years since the MEND (1993) report appeared, there has been some oscillation between the two approaches (oxic *vs*. anoxic control of pore-water metal concentrations), culminating in the recent emergence of a more nuanced appreciation that both sorption and precipitation reactions can intervene as the oxygenation of the surface sediment changes (e.g., seasonally or as a function of drought) and as the sediment ages.
- Linked to this general appreciation of the importance of pore-water metal concentrations as an estimate of the geochemical and biological availability of sediment-associated metals, considerable research has been devoted to understanding the post-depositional mobility of metals in sediments (reviewed in section 4.4). For several metals and oxyanions (e.g., Pb, Mo and As), there is evidence for their mobilization in the zone where reductive dissolution of iron oxyhydroxides occurs, and for their removal lower down in the sediment profile by precipitation as a solid sulphide. However, the overall influence of these diagenetic reactions on the metal concentration profiles in the sediment cores was negligible for most of the studied metals, and thus post-depositional mobility of the metal did not exert a significant effect on the observed solid-phase metal profiles in the sediment. Similar conclusions would also be expected for tailings, given the very high background concentration of metals in the solid phase.

Biomonitoring

In addition to the geochemical approaches described above, the earlier MEND reports also considered indigenous aquatic organisms, i.e. those living in the subaqueous tailings facility, and their use as biomonitor organisms; this information is updated in section 4.5 of the present report. In principle, properly chosen biomonitors could be used to determine if the metals present in the tailings are available to the indigenous plants and animals and to assess if the biomonitor organisms are metal-stressed or not. This can be done by sampling the indigenous fauna and flora at the site ('passive' biomonitoring) or by transplanting metal-naïve plants or animals into the water body and allowing them to interact with the tailings ('active' biomonitoring). In both approaches, there is an obvious need to have nearby reference sites that can be used for comparison purposes. The biomonitor species can be chosen to target either the tailings or the overlying water. An obvious advantage of the biomonitoring approach is that the bioaccumulated metal concentrations provide an estimate of the overall metal bioavailability in a given water body (integrating waterborne and diet-borne metals) – use of the organism itself obviates the need to estimate the 'bioavailability' of the metal in the water or in the ingested food. Measures of bioaccumulated metals have an additional potential advantage in that they can be used within the framework of a Tissue Residue Approach for toxicity assessment, where metal concentrations in whole organisms or specific organs are compared to threshold values that are known to trigger deleterious effects.

As a refinement to the Tissue Residue Approach, which normally deals with the total bioaccumulated metal concentration, it is also possible to consider the subcellular fate of the metal. In effect, the bioaccumulated metals may be detoxified, for example by sequestration in insoluble granules, by isolation in membrane-bound vesicles, or by complexation with cytosolic ligands. The form in which the bioaccumulated metal exists may also affect its fate if the plant or animal is consumed as food. Simple techniques that have the potential to differentiate between detoxified metals and biologically available metals are reviewed in section 4.5.2.

Parameters that have been used to detect metal exposure and potential effects in aquatic organisms include tissue metal concentrations (as described above), metallothionein concentrations and indicators of overall organism health, such as condition indices, growth or reproductive endpoints. Metal stress also may induce subtle effects at the subcellular level that do not necessarily translate into changes in these traditionally measured variables, but that may still have a detrimental metabolic cost. Several new ecotoxicological tools, introduced in the post-2008 period, are being used to probe for new biomarkers of metal-induced stress.

One of these tools is 'transcriptomics', the study of the 'transcriptome' of native organisms chronically exposed to metals in their environment. The transcriptome reflects the genes that are being expressed in the organism and thus will tend to vary as function of the ambient conditions and the organism's physiological state. In this context, the transcriptome can be considered as a potential addition to the small number of metal-sensitive 'biomarkers' that existed prior to 2008. Using this approach, laboratory-reared animals can be exposed to water or sediment obtained from the field and changes in their transcriptome can be interpreted as a response to bioavailable contaminants present in the field sample. In a variant of this bioassay approach, laboratory-raised test organisms (e.g., fathead minnows or amphipods) can be caged in the field, at reference sites and at sites that are subject to contaminant inputs; differences in their transcriptomes can then be examined. This use of the transcriptome to identify metal-induced deleterious effects is a promising but not yet mature approach.

Metal sensitivity of different organisms

Among the organisms that attempt to colonize or could be wanted in a given SAD facility, which species are the most sensitive to metals and the most likely to be adversely affected? Since some organisms may show marked sensitivity to one metal but tolerance to others, the answer to this question will vary from one ore body to another (or from one mine operation to another), as a function of the specific metals (and their form) present in the tailings. In addition, these differences in sensitivity to various metals are not necessarily the same for all target species. To address this question, the 'species sensitivity distributions' or SSDs were examined for many of the metals of interest, both essential and non-essential (section 5). For each metal, the most and least sensitive species have been compiled in Table 5-1; small crustaceans (e.g., daphnids, amphipods) and unicellular algae appear frequently in the 'most sensitive' column, whereas the 'least sensitive' column includes a greater variety of organisms. The limitations of the SSD approach are also explored in section 5.3.

Oxyanions

Arsenic (As), molybdenum Mo), antimony (Sb) and selenium (Se) were chosen as representative oxyanions, none of which were considered in the previous MEND reviews (1993; 2009). These elements share a number of properties: absence of cationic forms; existence as oxyanions; existence of multiple oxidation states. As a result, their geochemical behaviour differs markedly from that of the other (cationic) elements considered in the present review.

Changes between oxidation states, i.e. 'redox reactions', are often slow. For example, redox couples such as As(III/V) and Sb(III/V) are frequently not at equilibrium and individual species can be found at concentrations that differ markedly from those predicted thermodynamically. However, many redox reactions can be microbiologically catalyzed, in which case the reaction rate will be determined by microbial growth and metabolic activity. For trace concentrations at pH 8, the sequence of redox reactions follows the approximate order O_2 > NO_3- > Mn(IV) > Se(VI) > Fe(III) >As(V) > SO_4 ≈ Mo(VI).

Another commonality among this group of elements is the presence of methylated forms (for As, Sb and Se). These are not metal complexes, where the cation and the ligand are bound together by a coordinate bond, but rather true organometallic molecules where the non-metallic element 'M' and the methyl group are linked by covalent bonds (both as $M\text{-}O\text{-}CH_3$ bonds and direct $M\text{-}CH_3$ bonds). Because of the presence of a covalent bond, these methylated forms are much more stable than typical metal cation complexes. The elements also react with reduced sulphur in sediments (either to precipitate as sulphides or to adsorb onto authigenic sulphides), and in this case their behaviour mirrors that of the cationic metals considered in this review. This reaction with reduced sulphur tends to immobilize As, Sb and Mo. The biogeochemical behaviour of the oxyanions at the sediment-water or tailings-water interface has been summarized, in an element-by-element approach, in Sections 6.2 to 6.5.

In their interaction with living organisms, elements such as As, Mo, Sb and Se clearly do not conform to the Biotic Ligand Model (BLM), since they exist in natural waters as oxyanions and as neutral polyhydroxy species rather than as free metal cations. The mechanisms by which they are taken up by living organisms are less well understood than is the case for (essential) metal cations, but a number of known uptake routes are described in section 6, notably uptake via anion transporters present in the cell membrane (e.g., SeO_4^{2-} via a sulphate transporter; AsO_4^{3-} and SbO_4^{3-} via a phosphate transporter) and by aquaporins ($As(OH)_3^0$; $Sb(OH)_3^0$).

Subaqueous disposal facilities

The final part of the Literature Review includes a detailed compilation of the anticipated differences between a SAD facility and a natural water body, followed by a synthesis of the data generated from the assessment of SAD facilities in Canada and elsewhere (Section 7). Based on the available data, subaqueous disposal of unoxidized tailings has met the primary objectives of limiting sulphide oxidation to within a few millimetres or centimetres of the sediment-water interface and minimizing metal/oxyanion release. Where tailings predicted to be acid-generating under aerial conditions were flooded prior to significant oxidation, post-closure metal/oxyanion levels in SAD facilities in British Columbia meet discharge criteria set by the Ministry of Environment without additional mitigation or drainage treatment. However, evidence supporting this conclusion is from studies of relatively short duration; long-term studies of the effects of biological colonization are lacking. Where underwater disposal of mine tailings has been less successful (i.e., where marked remobilization of metal cations has been observed), water quality degradation has resulted from tailings oxidation during processing, an earlier extended period of sub-aerial exposure prior to flooding, or periodic exposure to the atmosphere due to water level changes (reviewed in Section 7.3). The success in limiting metal/oxyanion release, coupled with an apparent lack of attention to aquatic reclamation, has, however, resulted in limited monitoring or study of tailings diagenesis and biological colonization in the post-closure period; there proved to be a dearth of biomonitoring data for SAD facilities.

Recommendations

The recommendations presented in Section 8 and Appendix E provide guidance regarding recommended tools and methodologies to predict and/or monitor the biological and ecological effects of submerged tailings. This section is not designed to be a detailed guide to field or laboratory work, but rather should be viewed as a general outline of how to approach the challenge of monitoring and assessing possible biological effects of the subaqueous disposal of mine tailings. A site-specific, phased approach is recommended, focusing on the properties and processes of interest. Given the paucity of data for most existing SAD facilities, an initial survey followed by some exploratory sampling and analysis will undoubtedly be necessary. In addition, field trials should be carried out with a number of the monitoring techniques that have been recommended. These techniques have been shown to be useful in metal-contaminated lakes, but they haven't been field-tested on submerged tailings.

'Data and knowledge gaps' that were identified during the preparation of the present report, and during its subsequent review by the MEND steering committee, can also be found in Section 8. Chief among these was a general lack of information about the hydrobiology of SAD facilities, their inherent heterogeneity, possible impediments to becoming productive habitat, and their variability over time (diagenetic changes). To address this situation, it is recommended that a limited campaign of field measurements be carried out in a representative selection of SAD facilities, focusing on mine-made facilities where the tailings were not exposed sub-aerially before flooding or on the edge of the facility and where disposal of mine tailings ceased at least 5 years ago. Process chemicals (e.g., cyanide; xanthates) and thiosalts have been detected in active SAD facilities (i.e., in the period before mine closure), but little is known about the possible persistence of these substances once active disposal of mine tailings has ceased. Samples of the tailings pore water and from the overlying water column should be tested for the residual presence of such process chemicals.

Sommaire Exécutif

Contexte

La disposition subaquatique (DSA) de résidus miniers riches en sulfures dans des installations construites est l'une des nombreuses méthodes d'atténuation utilisées par les mines. Le principal mécanisme d'atténuation résultant de l'élimination sous l'eau est la limitation de la pénétration d'oxygène dans les pores remplis d'eau présents dans les résidus, ce qui réduit considérablement l'oxydation des sulfures, minimise la lixiviation des métaux et empêche le développement d'un drainage acide.

À ce jour, la plupart des études et activités de surveillance liées aux installations de DSA fermées se sont concentrées sur les performances physiques et géochimiques initiales des installations et sur la chimie résultante des eaux de surface retenues dans l'installation. Les aspects à long terme, tels que l'addition progressive de matières organiques naturelles par la sédimentation au-dessus des résidus et la performance biologique globale de ces installations, ne sont pas aussi bien compris. En particulier, une lacune majeure dans la compréhension concerne la colonisation biologique de telles installations, la santé des communautés biologiques établies et l'influence de ces communautés sur la géochimie des eaux et des sédiments.

Des revues de la littérature scientifique dans ce domaine général ont été commandées par le programme de neutralisation des eaux de drainage dans l'environnement minier (NEDEM) en 1993 et 2009. Ces études ont porté sur les moyens possibles d'évaluer les effets biologiques potentiels de la DSA des résidus miniers, en mettant l'accent sur la faune et la flore qui avaient colonisé le bassin de rétention des résidus. S'appuyant sur ces rapports antérieurs, la présente étude a été conçue (i) pour fournir des informations actualisées sur les changements diagénétiques potentiels dans les résidus immergés et sur les interactions biogéochimiques entre les résidus immergés et les communautés aquatiques sus-jacentes et (ii) sur la base des informations obtenues, pour fournir des indications sur les outils et méthodologies recommandés pour prévoir et / ou surveiller les effets biologiques des résidus immergés.

Les contaminants abordés dans le présent rapport se répartissent en deux catégories: (i) les oligo-éléments cationiques riches en données qui ont été traités dans les revues précédentes (Cd, Cu, Pb, Ni et Zn) et (ii) des oligo-éléments formant des oxyanions et des espèces polyhydroxy neutres, qui n'ont pas été traitées dans les rapports précédents (As, Mo, Sb, Se). D'autres éléments, tels que l'Al, le Fe et le Mn, ont été pris en compte dans le contexte des changements diagénétiques qui se produisent dans les sédiments et les résidus miniers après leur dépôt dans un parc à résidus subaquatiques.

Contenu

Le présent rapport comprend une introduction (Section 1), une revue de la littérature (Sections 2 à 7) et un ensemble de recommandations (Section 8). La revue de littérature examine d'abord comment les métaux dissous et ceux présents dans la nourriture interagissent avec les organismes vivants (Sections 2 et 3), puis intègre ces deux vecteurs d'absorption et considère la biodisponibilité des métaux dans les sédiments et les résidus miniers immergés (Section 4). Cette dernière section comprend également des exemples de différentes approches de biosurveillance pouvant être utilisées pour évaluer la biodisponibilité des métaux dans une installation DSA. La revue de la littérature porte également sur la sensibilité relative de différents organismes aquatiques aux métaux préoccupants (Section 5) et donne un aperçu de la chimie environnementale de divers oxyanions et de leur biodisponibilité (Section 6). La dernière partie de la revue de la littérature porte sur les données générées par l'évaluation de l'utilisation de la DSA au Canada et ailleurs, que l'on trouve dans les rapports des sociétés et des gouvernements et dans les comptes rendus de conférences (Section 7). La section des recommandations s'appuie sur toutes ces informations et fournit des indications sur les outils et méthodologies recommandés qui pourraient être utilisés pour évaluer les effets biologiques des résidus immergés.

Résultats

Outils

Dans les revues précédentes du programme NEDEM, différentes approches ou outils ont été présentés, notamment: (i) le modèle du ligand biotique (BLM) pour évaluer la biodisponibilité des métaux présents dans l'eau sus-jacente ou dans les eaux interstitielles des résidus; (ii) le modèle de sulfures amorphes réactifs – métaux extraits simultanément (AVS – SEM) pour évaluer la toxicité potentielle des métaux présents dans les résidus immergés depuis longtemps et anoxiques; et (iii) des modèles d'équilibres d'adsorption de surface pour calculer les concentrations de cations libres dans les eaux interstitielles des résidus oxiques.

- Le BLM était déjà raisonnablement arrivé à maturité en 2008 et l'approche a été considérablement améliorée depuis lors, notamment en ce qui concerne sa capacité à prédire la toxicité chronique (améliorations au module de spéciation métallique) et son application aux mélanges métalliques. La revue du rapport NEDEM (2009) demeure un résumé précis et utile, en particulier pour les métaux bien étudiés (Cd, Cu, Ni, Pb, Zn). Pour évaluer la biodisponibilité de ces métaux, sous forme dissoute (c'est-à-dire dans l'eau qui recouvre les résidus immergés et dans l'eau interstitielle résidant dans les interstices des résidus), l'approche BLM reste l'outil recommandé à utiliser.
- Le modèle AVS – SEM et le modèle d'équilibres d'adsorption sont tous deux conçus pour fournir des estimations de la concentration en cations métalliques libres dans les eaux interstitielles des sédiments ou des résidus, cette concentration étant reconnue comme le meilleur moyen d'évaluer la biodisponibilité d'un métal associé aux sédiments. Le premier modèle s'applique aux conditions anoxiques et suppose que les concentrations de métaux interstitiels sont contrôlées par des réactions de précipitation-dissolution avec des sulfures amorphes réactifs (AVS), alors que le second modèle est étalonné pour des conditions oxiques et considère que les concentrations de métaux interstitiels sont contrôlées par des réactions de sorption sur des sorbants tels que les oxyhydroxydes de Fe et de Mn ou la matière organique particulaire des sédiments. Au cours des 20 années écoulées depuis la parution du rapport NEDEM (1993), il y a eu un va-et-vient entre ces deux approches (contrôle oxique ou contrôle anoxique des concentrations des métaux interstitiels), aboutissant à l'émergence récente d'une appréciation plus nuancée, qui accepte que l'importance relative des deux types de réaction (adsorption *vs* précipitation comme sulfures) puisse varier en fonction du vieillissement des sédiments et de leur degré d'oxygénation (ex. : variabilité saisonnière ou en fonction de la sécheresse).
- En lien avec cette appréciation générale de l'importance des concentrations de métaux interstitiels en tant qu'estimation de la disponibilité géochimique et biologique des métaux associés aux sédiments, des efforts de recherche considérables ont été consacrés à la compréhension de la mobilité post-dépositionnelle des métaux dans les sédiments (Section 4.4). Pour plusieurs métaux et oxyanions (ex. : Pb, Mo et As), il existe des indices de leur mobilisation dans la zone où se produit la dissolution réductrice des oxyhydroxydes de fer et leur élimination plus basse dans le profil sédimentaire par précipitation sous forme de sulfures solides. Cependant, l'influence globale de ces réactions diagénétiques sur les profils de concentration en métal dans les carottes de sédiments était négligeable pour la plupart des métaux étudiés et la mobilité post-dépositionnelle du métal n'a donc pas d'effet significatif sur les profils métalliques observés dans la phase solide des sédiments. Des conclusions similaires seraient également attendues pour les résidus miniers, compte tenu de la concentration naturelle très élevée des métaux dans la phase solide.

Biosurveillance

Outre les approches géochimiques décrites ci-dessus, les rapports NEDEM antérieurs prenaient également en compte les organismes aquatiques indigènes, c'est-à-dire ceux vivant dans le parc à résidus subaquatiques, et leur utilisation en tant que biomoniteurs; cette information est mise à jour dans la Section 4.5 du présent rapport. En principe, des biomoniteurs correctement choisis pourraient être utilisés pour déterminer si les métaux présents dans les résidus sont disponibles pour les plantes et les animaux indigènes et pour évaluer si ces organismes sont stressés par des métaux ou non. Cela peut être fait par échantillonnage de la faune et de la flore indigènes au site (biosurveillance « passive ») ou par transplantation de plantes ou d'animaux 'propres' (n'ayant jamais été exposés à des concentrations élevées en métaux) dans le parc à résidus subaquatiques et en leur permettant d'interagir avec les résidus (biosurveillance « active »). Dans les deux approches, il existe un besoin évident d'avoir des sites de référence à proximité pouvant être utilisés à des fins de comparaison. Les espèces de biomoniteurs peuvent être choisies pour cibler les résidus ou les eaux sus-jacentes. Un avantage évident de l'approche de biosurveillance est que les concentrations de métaux bioaccumulés fournissent une estimation de la biodisponibilité métallique globale dans un plan d'eau donné (intégrant les métaux dissous et les métaux associés à la nourriture) – l'utilisation de l'organisme lui-même élimine la nécessité d'estimer la « biodisponibilité » du métal dans l'eau ou dans la nourriture ingérée. Les mesures de métaux bioaccumulés ont un avantage potentiel supplémentaire en ce sens qu'ils peuvent être utilisés pour une évaluation de la toxicité, où les concentrations de métaux dans des organismes entiers ou des organes spécifiques sont comparées aux valeurs de seuil qui sont connues pour déclencher des effets délétères.

Pour affiner cette approche de « résidus tissulaires », qui porte normalement sur la concentration totale en métaux bioaccumulés, il est également possible de tenir compte du devenir subcellulaire du métal. En effet, les métaux bioaccumulés peuvent être détoxiqués, par exemple par séquestration dans des granules insolubles, par isolement dans des vésicules membranaires ou par complexation avec des ligands cytosoliques. La forme sous laquelle le métal bioaccumulé existe peut également affecter son devenir si la plante ou l'animal est consommé comme aliment. Les techniques simples susceptibles de différencier les métaux détoxiqués des métaux biodisponibles sont examinées à la Section 4.5.2.

Les paramètres qui ont été utilisés pour détecter l'exposition aux métaux et leurs effets potentiels chez les organismes aquatiques comprennent des concentrations tissulaires de métaux (comme décrit ci-dessus), les concentrations de métallothionéine et les indicateurs de la santé générale de l'organisme, comme les indices de condition, la croissance ou le succès de la reproduction. Le stress des métaux peut également induire des effets subtils au niveau subcellulaire qui ne se traduisent pas nécessairement par des modifications de ces variables traditionnellement mesurées, mais qui peuvent néanmoins avoir un coût métabolique préjudiciable. Plusieurs nouveaux outils écotoxicologiques, introduits après 2008, sont utilisés pour détecter de nouveaux biomarqueurs du stress induit par les métaux.

L'un de ces outils est la « transcriptomique », l'étude du « transcriptome » d'organismes indigènes exposés de façon chronique aux métaux dans leur environnement. Le transcriptome reflète les gènes qui sont exprimés dans l'organisme et tendra donc à varier en fonction des conditions ambiantes et de l'état physiologique de l'organisme. Dans ce contexte, le transcriptome peut être considéré comme un ajout potentiel au petit nombre de « biomarqueurs » sensibles aux métaux qui existaient avant 2008. En utilisant cette approche, les animaux élevés en laboratoire peuvent être exposés à l'eau ou les sédiments provenant du terrain et les changements dans leur transcriptome peuvent être interprétés comme une réponse aux contaminants biodisponibles présents dans l'échantillon récolté sur le terrain. Dans une variante de cette approche d'essai biologique, les organismes de test élevés en laboratoire (par exemple, vairons ou amphipodes) peuvent être mis en cage sur le terrain, sur les sites de référence et sur les sites qui sont soumis à des contaminants intrants; les différences dans leurs transcriptomes peuvent alors être examinées. Cette utilisation du transcriptome pour identifier les effets délétères induits par le métal est une approche prometteuse, mais pas encore mature.

Sensibilité aux métaux de différents organismes

Parmi les organismes qui tentent de coloniser des installations de DSA, ou qui seraient recherchés dans une telle installation, quelles sont les espèces les plus sensibles aux métaux et les plus susceptibles d'être affectées négativement? Étant donné que certains organismes peuvent montrer une sensibilité marquée à un métal, mais une tolérance à d'autres, la réponse à cette question variera d'un gisement à l'autre (ou d'une exploitation à l'autre), en fonction de la nature et la forme des métaux présents dans les résidus miniers. De plus, ces différences de sensibilité à divers métaux ne sont pas nécessairement les mêmes pour toutes les espèces cibles. Pour répondre à cette question, les « distributions de sensibilité des espèces » (« species sensitivity distributions » en anglais, ou SSDs) ont été examinées pour plusieurs des métaux d'intérêt, à la fois essentiels et non essentiels (Section 5). Pour chaque métal, les espèces les plus sensibles et les moins sensibles ont été rassemblées dans le tableau 5-1; petits crustacés (ex. : daphnies, amphipodes) et algues unicellulaires apparaissent fréquemment dans la colonne «plus sensible», alors que la colonne «moins sensible» comprend une plus grande variété d'organismes. Les limites de l'approche SSD sont également explorées dans la Section 5.3.

Oxyanions

L'arsenic (As), le molybdène (Mo), l'antimoine (Sb) et le sélénium (Se) ont été choisis comme oxyanions représentatifs; aucun de ces éléments n'a été pris en compte dans les revues précédentes du NEDEM (1993; 2009). Ces éléments partagent un certain nombre de propriétés: absence de formes cationiques; existence comme oxyanions; présence de multiples états d'oxydation. En conséquence, leur comportement géochimique diffère sensiblement de celui des autres éléments (cationiques) considérés dans la présente revue.

Les changements entre les états d'oxydation, c'est-à-dire les réactions d'oxydoréduction, sont souvent lents. Par exemple, les couples rédox tels que As (III / V) et Sb (III / V) ne sont souvent pas à l'équilibre et des espèces individuelles peuvent être trouvées à des concentrations très différentes de celles prédites thermodynamiquement. Cependant, de nombreuses réactions rédox peuvent être catalysées microbiologiquement, auquel cas la vitesse de réaction sera déterminée par la croissance microbienne et l'activité métabolique. Pour les concentrations en traces à pH 8, la séquence des réactions rédox suit l'ordre approximatif O_2> NO_3^-> Mn (IV)> Se (VI)> Fe (III)> As (V)> SO_4 ≈ Mo (VI).

Une autre caractéristique commune à ce groupe d'éléments est la présence de formes méthylées (pour As, Sb et Se). Ce ne sont pas des complexes métalliques, où le cation et le ligand sont liés entre eux par une liaison coordonnée, mais plutôt de véritables molécules organométalliques où l'élément non métallique « M » et le groupe méthyle sont liés par des liaisons covalentes (comme des liaisons $M-O-CH_3$ ou des liaisons directes $M-CH_3$). En raison de la présence d'une liaison covalente, ces formes méthylées sont beaucoup plus stables que les complexes de cations métalliques typiques. Ces éléments réagissent également avec le soufre réduit dans les sédiments (soit pour précipiter sous forme de sulfures ou pour s'adsorber sur des sulfures authigènes), et dans ce cas leur comportement ressemble à celui des métaux cationiques considérés dans cette revue. Cette réaction avec le soufre réduit a tendance à immobiliser As, Sb et Mo. Une synthèse du comportement biogéochimique des oxyanions à l'interface eau-sédiment ou eau-résidus, dans une approche élément par élément, se trouve dans les Sections 6.2 à 6.5.

Dans leur interaction avec des organismes vivants, des éléments tels que As, Mo, Sb et Se ne se conforment manifestement pas au modèle du ligand biotique (BLM), car ils existent dans les eaux naturelles sous forme d'oxyanions et d'espèces polyhydroxy neutres et non comme cations libres. Les mécanismes par lesquels ils sont absorbés par les organismes vivants sont moins bien compris que ceux des cations métalliques (essentiels), mais un certain nombre de voies d'absorption connues sont décrites dans la Section 6, notamment l'absorption par des transporteurs anioniques présents dans la membrane cellulaire (par exemple, SeO_4^{2-} via un transporteur de sulfate; AsO_4^{3-} et SbO_4^{3-} via un transporteur de phosphate) et par des aquaporines ($As(OH)_3^0$; $Sb(OH)_3^0$).

Installations de disposition subaquatique (DSA)
La dernière partie de la revue de la littérature comprend une compilation détaillée des différences anticipées entre une installation de DSA et un plan d'eau naturel, suivie d'une synthèse des données générées par l'évaluation des installations de DSA au Canada et ailleurs (Section 7). D'après les données disponibles, l'élimination sous l'eau des résidus miniers non oxydés a permis de limiter l'oxydation des sulfures à quelques millimètres ou centimètres de l'interface eau / sédiments et de réduire au minimum la libération de métaux et d'oxyanions. Dans les cas où des résidus potentiellement générateurs d'acide dans des conditions aériennes ont été placés dans des installations DSA avant d'avoir subi une oxydation importante, les concentrations aqueuses de métaux et d'oxyanions dans ces installations, localisées en Colombie-Britannique et ne recevant plus de rejets, répondent aux critères de rejet définis par le ministère provincial de l'Environnement, sans traitement supplémentaire. Cependant, les preuves à l'appui de cette conclusion proviennent d'études de durée relativement courte; les études à long terme font défaut.

Lorsque la disposition sous l'eau des résidus miniers a été moins fructueuse (c'est-à-dire, lorsqu'une remobilisation marquée des cations métalliques a été observée), la dégradation de la qualité de l'eau était attribuable à l'oxydation des résidus pendant le traitement à l'usine, à leur exposition préalable à l'atmosphère avant leur immersion, ou encore à leur exposition périodique à l'oxygène due aux variations du niveau d'eau du bassin (discuté dans la Section 7.3). Le succès rencontré en ce qui concerne le respect des critères de rejet applicables, associé à un manque apparent d'attention à la réhabilitation du site, a toutefois entraîné une surveillance limitée de la diagenèse des résidus et de la colonisation biologique après la fermeture. Il y a une pénurie marquée de données de biosurveillance pour les installations DSA.

Recommandations
Les recommandations présentées à la Section 8 et à l'Annexe E fournissent des indications sur les outils et méthodologies recommandés pour prévoir et / ou surveiller les effets biologiques et écologiques des résidus immergés. Cette section n'est pas conçue pour être un guide détaillé des travaux sur le terrain ou en laboratoire, mais devrait plutôt être considérée comme un aperçu général de la manière de surveiller et d'évaluer les effets biologiques possibles de la disposition sous l'eau des résidus miniers. Une approche spécifique à chaque site et procédant par étapes est recommandée, en mettant l'accent sur les propriétés et les processus d'intérêt. Étant donné le manque de données pour la plupart des installations de DSA existantes, une enquête initiale suivie de quelques échantillonnages et analyses exploratoires sera sans aucun doute nécessaire. En outre, des essais sur le terrain devraient être effectués avec un certain nombre de techniques de surveillance recommandées. Ces techniques se sont révélées utiles dans les lacs contaminés par les métaux, mais elles n'ont pas été testées sur le terrain avec des résidus immergés.

Les lacunes en matière de données et de connaissances, identifiées lors de la préparation du présent rapport et lors de son examen ultérieur par le comité directeur du programme NEDEM, figurent également à la Section 8. Parmi celles-ci, citons le manque général d'informations sur l'hydrobiologie des installations DSA, leur hétérogénéité inhérente, les obstacles possibles à la création d'un habitat productif et leur variabilité dans le temps (changements diagénétiques). Pour remédier à cette situation, il est recommandé de mener une campagne limitée de mesures sur le terrain dans une sélection représentative d'installations de DSA, en mettant l'accent sur les installations minières où les résidus n'ont pas été exposés à l'atmosphère avant d'être immergés ou aux abords de l'installation lors de sécheresses, et dans les installations où l'ajout des résidus miniers a cessé il y a au moins 5 ans. Des produits chimiques de transformation (ex. : cyanures, xanthates) et des thiosels ont été détectés dans les installations de DSA actives (avant la fermeture de la mine), mais on sait peu de choses sur la persistance éventuelle de ces substances. Des échantillons de l'eau interstitielle des résidus et de la colonne d'eau sus-jacente doivent être testés pour déterminer si ces produits chimiques sont toujours présents.

1 Introduction

1.1 Overview

The initial studies on the subaqueous disposal of mine tailings, conceived as a means to minimize their oxidation and prevent elevated metal release and the development of ARD, were conducted under the auspices of the Mine Environment Neutral Drainage (MEND) programme (a search of the MEND publications database with 'Subaqueous disposal' as a key word identified more than 100 reports). Demonstration of the geochemical efficacy of this approach led to its adoption by several Canadian mines. The mines that have adopted subaqueous disposal of their tailings are those where the metal-bearing ore is sulphide-rich (e.g., Ag, Au, Cu, Ni, Zn). In most cases, the subaqueous disposal facilities are constructed above-ground but there is also disposal in pit-lakes and occasionally natural lakes. In the past, mines frequently placed tailings at least partially in natural water bodies but this practice is now only allowed by under special circumstances the Federal Fisheries Act (e.g., Eskay Creek, Brucejack and Voisey's Bay mines).

Subaqueous disposal (flooding) in constructed facilities is one of a number of mitigation methods used by mines. The primary mitigation mechanism resulting from subaqueous disposal is prevention of oxygen ingress, which almost completely prevents oxidation from transforming sulphides into compounds that can dissolve, minimizing metal leaching and preventing acidic drainage. Even under less than ideal conditions, subaqueous disposal results in a thousand times reduction in the rate of sulphide oxidation (Price 2001). Where successful, subaqueous disposal creates productive habitat and reduces closure costs to the relatively minor costs of maintaining dams and other water management features (Price 2013).

Like other mitigation measures, subaqueous tailings facilities must be designed, constructed, operated, maintained and closed in a manner that ensures that long-term receiving environment and reclamation objectives can be met during the closure and post-closure phases of the mine life cycle (Price and Errington 1998). After deposition into the tailings facility stops, natural hydrological and ecological processes will intervene. Flora and fauna may become established, with the result that the subaqueous tailings facility can become an important ecological component, contributing habitat, food and drainage. Over time, many of these sites have become colonized by a variety of aquatic organisms such as aquatic plants, benthic invertebrates, fish and piscivorous wildlife. Consequently, the following question has arisen: How do these aquatic communities compare to natural environments, is their performance in some manner affected by the submerged tailings, and is some supplemental form of mitigation required? For example, elevated concentrations of metals and trace elements present in the tailings could have an effect on the resident aquatic organisms. Alternatively, the physical properties of the tailings (e.g., grain-size distribution) or a scarcity of certain key components (e.g., inorganic nutrients; natural organic matter) may impede colonization and limit the development of the aquatic community.

To date, most studies and monitoring activities related to closed subaqueous tailings facilities have focused on the initial physical and geochemical performance of the facilities and the resulting chemistry of the surface water, and accordingly the diagenesis and the long-term biological performance of such facilities are not as well understood. In particular, a major gap in understanding relates to the biological colonization of such facilities, the health of biological communities that are established, and the influence of those communities on ecological performance and water and sediment geochemistry.

1.2 Study Objectives

The objectives of the present study were (i) to conduct a literature review and provide updated information on potential diagenetic changes in submerged tailings and on biogeochemical interactions between submerged tailings and overlying aquatic communities, and (ii) based on the information obtained in part (i), to provide guidance regarding recommended tools and methodologies to be used to predict and/or monitor the biological and ecological effects of submerged tailings.

Two earlier MEND reports addressed possible means of evaluating the biological effects of subaqueous disposal of mine tailings (MEND 1993; MEND 2009). Various approaches were discussed, including the following:

- Use of the 'Biotic Ligand Model' or BLM[1] (Paquin et al. 2002) to evaluate the bioavailability of metals present in the overlying water.
- Use of the 'AVS – SEM model' (Ankley 1996; Ankley et al. 1996) to evaluate the potential toxicity of the metals present in the submerged, aged and anoxic tailings.
- Use of sorptive equilibrium models (Campbell and Tessier 1996) to calculate the free metal ion concentrations in the oxic tailings pore waters.
- Use of indigenous aquatic organisms (Phillips and Rainbow 1993), i.e. those living in the subaqueous tailings facility, as biomonitor organisms and determine if they are metal-stressed or not.

The present report should be read in conjunction with the earlier ones (MEND 1993; MEND 2009). The study provided here specifically considers data available to the end of 2017, eight years after the last update, including:

- Data generated from the assessment of subaqueous disposal facilities across Canada in company and government reports and conference proceedings (as furnished with assistance by NRCan-CanmetMINING).
- Studies published in the peer-reviewed scientific literature since 2008, with an emphasis on the four 'tools' listed above: BLM; SEM – AVS; sorptive equilibrium models; indigenous biomonitor organisms.

In this context, the following questions have been addressed:

- How is long-term water quality of the water column influenced by submerged tailings?
- What is the bioavailability of metals in the subaqueous tailings, which may or may not be covered with natural sediments?
- What is the potential for bioaccumulation of contaminants and trophic transfer through the freshwater food chain?
- How might the biology and ecology of the system with subaqueous tailings differ from natural water bodies in similar hydrological, geological and climatological settings?

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[1] For the definition of this and other acronyms, please consult the Glossary.

- What organisms are most sensitive and likely to be adversely affected, including aquatic organisms and non-aquatic organisms that feed on, reside in, or transit through the system?
- How do the physical and chemical properties of the subaqueous tailings affect the natural colonization of the tailings facility?
- What is the effect of colonizing flora and fauna on the physical and chemical properties of the subaqueous tailings, and have they or will they modify the release of contaminants into the water cover?
- Are there confounding factors to consider (e.g., lack of O_2 under ice cover; scouring of littoral areas by ice during spring snowmelt period)?
- What is recommended for future studies?

An update on the four 'tools' is provided, based on a review of relevant papers published in the peer-reviewed literature since the MEND Report 2.11.2(b) (MEND 2009) appeared, and several new 'tools' that have been introduced in the post-2008 period (e.g., environmental DNA; toxicogenomics) are described.

Contaminants addressed in the present preview fall into two categories: (i) the data-rich cationic trace elements that were covered in the previous reviews (Cd, Cu, Pb, Ni and Zn) and (ii) elements forming oxyanions and neutral polyhydroxy species that were not covered in the previous reviews (As, Mo, Sb, Se). Other elements, such as Al, Fe and Mn, have been considered in the context of the diagenetic changes that occur in sediments and tailings after their deposition in a subaqueous tailings facility. Mercury has not been considered, in part because of its lack of prominence in mined rock, but also because its biogeochemical behaviour is very different from that of other metals and oxyanions. Extensive and recent reviews of Hg biogeochemistry are available in the post-2009 scientific literature, including Selin (2009); Scheuhammer et al. (2012); Bessinger (2014); Conder et al. (2015) and ECCC (2016).

2 Metal – Organism Interactions (water)

As mentioned earlier, a number of subaqueous disposal facilities have been established in Canada. These facilities have been subject to periodic control of the quality of the surface water within the facility, and in general the quality of water overlying the submerged tailings typically meets regulatory limits intended to be protective of the downstream environment. However, although the waters discharged from a subaqueous disposal site may meet regulatory criteria, these limits were not intended for and may not be sufficiently stringent to protect aquatic life within the facility – one of the main objectives of the present report is to identify methods that could be used to assess the state of the biota that succeed in colonizing a subaqueous disposal facility. Until recently the regulatory limits have been expressed in terms of total metal concentrations or total dissolved metal concentrations, for individual metals.

Spurred in large part by the environmental risk assessments carried out in the European Union for various metals (e.g., Cu, Ni, Pb, Zn), there has been a world-wide effort to develop and apply regulatory limits for metals that consider metal speciation and 'bioavailability'. For surface waters, and to a lesser extent for sediment pore waters, this effort has been based largely on the Biotic Ligand Model or BLM.

2.1 Brief description of the BLM

It is useful to distinguish between the BLM concept and BLM models. The BLM concept is based on two fundamental ideas: (i) metal cations normally cross epithelial biological membranes[2] and enter living organisms via facilitated transport; and (ii) this trans-membrane transport occurs relatively slowly, such that an equilibrium is established between the external (exposure) environment and the biological surface (Figure 2-1, mechanism #1). In the case of a unicellular alga or bacterium, the epithelial membrane would be the plasmalemma or cell membrane, whereas for a higher organism it might be a gill or olfactory membrane.

For a given pH and water hardness (and assuming that mechanisms 2 and 3 in Figure 2-1 are not operative), the biological response to the metal will vary as a function of the concentration of the metal that is bound to the membrane transporters, and this concentration will in turn vary as a function of the free metal ion concentration in the surrounding water. If the pH or the hardness of the water varies, the concentration of metal bound to the membrane transporters may be affected; these cases are usually treated as examples of competition between the free metal ion (M^{z+}) and the proton (H^+) or the hardness cations (Ca^{2+}, Mg^{2+}) for binding to the membrane transporters (e.g., at higher hardness, there is more competition for binding to the membrane transporter, less metal bound to the transporter, and thus a reduced biological response).

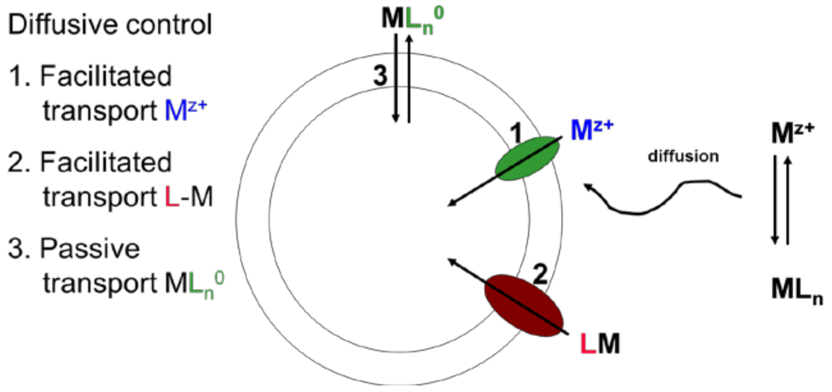

Figure 2-1: Metal-organism interactions

M^{z+}= metal cation; M-L_n = metal-ligand complex; M-L_n^0 = neutral lipophilic metal-ligand complex; L-M = metal bound to an assimilable ligand.

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[2] In this context, ‘epithelial membrane’ refers to a biological membrane that separates a living organism from its abiotic environment.

It follows that the biological response should be predictable if the concentration of the free metal ion in the exposure medium can be measured or calculated, and if the competitive effects of the proton and the hardness cations can be taken into account quantitatively. In effect, this is what the various BLM models do – they include a *speciation module* that takes the known water chemistry and calculates the free metal ion concentration, and a *biological interaction module* that takes into account the binding of M^{Z+}, H^+ and other cations (e.g., Ca^{2+}, Mg^{2+}) to the so-called 'biotic ligand'[3]. The speciation module will be same for all organisms, but the biological interaction module will vary from one organism to another (reflecting, in part, the varying tolerances that aquatic organisms have to a given metal exposure).

The BLM approach has been used to 'harmonize' much of the literature on the aquatic toxicology of cationic metals. This harmonization has involved what the practitioners refer to as 'bioavailability normalization', meaning that they take the results of toxicity tests carried out on the same organism, in different laboratories and in waters of different composition, and express the results on a common basis, as the amount of metal calculated to have bound to the biotic ligand (MERAG 2014). Proceeding in this manner results in much less dispersion around the calculated EC_{50} or LC_{50} concentrations. For example, De Schamphelaere et al. (2002) tested the toxicity of Cu to *Daphnia magna* in 11 different European Union surface water samples and reported that the 48-h EC_{50} value, expressed as dissolved Cu, varied 23-fold (34 to 790 µg Cu/L); in contrast, the BLM predictions of EC_{50} values in these same waters varied by a factor of ≤ 2.

This is a simplified description of the BLM approach. Additional details can be found in Paquin et al. (2002); Wilkinson and Buffle (2004); Campbell and Fortin (2013). Table A-1 in Appendix A summarizes known exceptions to the BLM.

2.2 BLM update

In the following update section, we will consider (i) improvements to the metal speciation calculations; (ii) potential problems with synthetic organic ligands; (iii) organism acclimation to antecedent exposure conditions; (iv) applications to metal mixtures; (v) applications to trivalent metals; and (vi) analytical methods of use in estimating metal bioavailability.[4]

2.2.1 Metal speciation calculations

Since 2008, the BLM *metal speciation module* has been substantially refined. For most publicly available BLM models, the speciation module is based on the Windermere Humic Aqueous Model or WHAM, the most recent version of which is Model VII (Tipping et al. 2011). Early versions of WHAM were calibrated against laboratory metal titrations of isolated natural organic matter, but subsequently considerable effort was expended to test and improve the ability of WHAM to predict free metal ion concentrations in

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[3] The term 'biotic ligand' refers to a metal-binding moiety or ligand that is an integral part of a living organism. Often it will correspond to a metal transporter or a metal-sensitive site, both present in an epithelial membrane and thus available for interaction with a waterborne metal.

[4] The BLM deals with metal-induced biological effects but not with metal adsorption on biological surfaces. Adsorption, a key process in controlling dissolved metal concentrations in oxic environments, can be modelled with surface complexation models (Section 4.3).

natural waters (Unsworth et al. 2006; Lofts and Tipping 2011; Mueller et al. 2012; Ahmed et al. 2013; Chappaz and Curtis 2013; Tipping et al. 2016). This is the area where progress on the BLM has been most evident, with improved handling of (i) metal complexation by dissolved organic carbon (DOC) at low metal concentrations and (ii) variations in the molecular nature of the DOC). It should also be noted here that the agreement between observed metal speciation and metal speciation as predicted by WHAM or other chemical equilibrium models is consistently better at moderate to high total dissolved metal concentrations than at the very low concentrations that prevail in pristine waters (Lofts and Tipping 2011). This observation is reassuring from a metal ecotoxicology perspective, given that waters with moderate to high metal concentrations will be those of primary concern.

2.2.2 Synthetic organic ligands

The BLM assumes that the organic ligands present in a given water sample can be modelled as a mixture of fulvic and humic acids. Normally this is a reasonable assumption but it can be confounded by the presence of strong synthetic organic ligands in waters that are affected by municipal or industrial discharges. This issue was mentioned in the MEND (2009) report and several examples have appeared in the literature (Baken et al. 2011; Turpin-Nagel and Vadas 2016). Mine process waters often contain organic reagents used in ore processing that could conceivably affect metal speciation and bioavailability. Following mine closure, and the cessation of municipal/industrial inputs to subaqueous disposal facilities, these synthetic organic ligands would be subject to photo- and bio-degradation and their influence would be predicted to diminish. This point is also discussed in Section 7.2

2.2.3 Organism acclimation

Within the *biological interaction module*, a potential limitation of the BLM approach is that the properties of the 'biotic ligand' are considered to be constant, meaning that both the number of epithelial binding sites and their affinity for the metal of interest (and for H^+, Ca^{2+} and Mg^{2+}) are invariable, allowing the interactions of the metal cation with the biological surface to be predicted accurately. However, there are examples in the literature that suggest this is not always the case, i.e., that organisms that are chronically exposed to a metal may acquire tolerance to the metal, and that this tolerance is linked to changes at the biological surface (fish – Niyogi and Wood (2004); daphnids – Muyssen and Janssen (2004); Muyssen et al. (2005); Muyssen and Janssen (2005); phytoplankton – Lavoie et al. (2012)). This variable was mentioned in the MEND (2009) report as an emerging issue but further work in the area has been limited, probably because if tolerance is acquired, the BLM will overestimate metal toxicity and thus the BLM approach is 'conservative' from a regulator's point of view.

2.2.4 Metal mixtures

In the real world, aquatic organisms are often chronically exposed to metal mixtures. Environmental regulators have tended to acknowledge the occurrence of metal mixtures in the receiving environment, but have then dealt with metals singly. The BLM is designed to treat one metal and one organism at a time, but in principle it could be adapted to consider several metals simultaneously.

There has been a recent surge of interest in how to deal with metal mixtures, driven in part by the Water Framework Directive in the European Union and the need to evaluate the overall health of EU surface waters. The overall objectives of these activities have been (i) to compare the results generated by the various metal-mixture toxicity models using common datasets, (ii) to gain a better understanding of chronic effects from metal-mixture exposures, and (iii) to develop mechanistic justification for the underlying assumptions of metal interactions at the 'biotic ligand'. These efforts have included carefully designed experiments and elaborate modelling exercises.

A useful introduction to the metal mixture question can be found in Meyer et al. (2015), who provide a guide to the sometimes confusing terminology and discuss the regulatory framework, qualitative and quantitative concepts, experimental designs for studying chemical mixtures, and methods for data analysis and visualization, with an emphasis on bioavailability and metal-metal interactions in mixtures of waterborne metals. This paper serves as an introduction to a series of 11 papers published in the special section of the *Environmental Toxicology and Chemistry* journal (MMME or Metal Mixtures Modelling Exercise: Farley and Meyer (2015); Farley et al. (2015); Meyer et al. (2015); Van Genderen et al. (2015). Several other reports have examined the literature on metal mixture toxicity, to evaluate the prevalence of 'greater than additive' or 'less than additive' toxicity (Wu et al. 2016; Liu et al. 2017b).

Current metal-mixture toxicity models incorporate the basic principles of the BLM. They use chemical speciation calculations to compute free ion activities of metals and major cations, and provide an evaluation of competing binding of metals and major cations to one or more binding sites on an organism using conventional competitive equilibrium chemistry. A single binding site implies that the different metals have the same mode of entry to the organism, whereas multiple binding sites introduce the concept of independent modes of entry. These chemical considerations are then linked to metal toxicity by correlating accumulated metal to toxicity using potency factors or toxicity-response functions.

One of the important conclusions from the MMME exercise cited above was that models calibrated from single-metal exposures provided reasonable predictions of metal mixture toxicity, using concentration-additive or independent action approaches (Van Genderen et al. 2015). Indeed, a consensus appears to be emerging that the assumption of additive toxicity is normally conservative and that examples of 'synergistic' or greater than additive responses to metal mixtures are rare. Preliminary indications are that these latter examples of greater than additive responses tend to occur only with certain metal combinations (e.g., those involving Cd).

2.2.5 Applications to trivalent metals

Almost all of the experimental tests of the BLM have been performed with divalent cations. Among the rare tests that have been carried out on trivalent metals such as Al^{3+}, Sc^{3+}, Cr^{3+} or the lanthanides, there are some noted discrepancies indicating that the metal is much more bioavailable than would have been predicted on the basis of its free-metal ion concentration (Wilkinson et al. 1990; Parent and Campbell 1994; Crémazy et al. 2013; Crémazy et al. 2014).

Trivalent cations tend to behave as 'hard' cations[5], showing a marked preference for binding to such ligands as the hydroxide (HO^-) and fluoride (F^-) anions, rather than to amine or reduced sulphur donor atoms. The experimental results suggest that the resulting complexes (e.g. $Al(OH)^{2+}$ and AlF^{2+}) are in

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[5] 'Hard' cations, as defined in the *Hard Acids and Bases* model, are those that are small, with high charge states and low polarizability (e.g., Na, K, Ca, Mg, Al, Fe). When forming complexes, hard cations show a strong preference for ligands with oxygen-bearing functional groups.

some way ‘bioavailable’, which is a radical departure from the BLM premise that only the free-metal ion can traverse an epithelial biological membrane. Since these metals are not known to be biologically essential, they must enter biological cells by ‘fooling’ the membrane transporters that normally transport monovalent and divalent metals. Even iron, present in both the Fe^{2+} and Fe^{3+} states in natural waters, is normally first reduced to the ferrous state before it traverses epithelial membranes. It is tempting to suggest that complexes such as $M(OH)^{2+}$ and MF^{2+} may masquerade as divalent cations and gain entry to living cells in this manner, but this idea remains speculative. The take-home message is that the BLM should be applied to trivalent metals with circumspection, particularly at circumneutral pH values that favour the formation of hydroxy-complexes.

2.2.6 Analytical methods of use in estimating metal bioavailability

As described above, BLMs use the total dissolved metal concentration as an input variable, together with ancillary water chemistry data (e.g., pH, [Ca], [Mg], [DOC]), and produce estimates of the metal’s bioavailability. In principle, it would also be possible to determine the metal’s speciation analytically, and use this value to predict the bioavailability of the metal. A very brief overview of possible analytical approaches can be found in Table 2-1.

Given the extensive experimental data that indicate that the free metal ion concentration ($[M^{z+}]$) is the best predictor of the bioavailability of waterborne metals (Campbell 1995), it would make sense to prioritize techniques that yield estimates of $[M^{z+}]$. The least ambiguous of these would involve the use of an ion-selective electrode (ISE), but unfortunately such electrodes are relatively insensitive; only in the case of copper have such measurements proved feasible in natural waters. A newer electrochemical technique (AGNES – see Table 2-1) is more sensitive and shows promise for determining free Cd, In, Pb and Zn. Equilibrium ion-exchange has also yielded useful results for free Cd, Cu, Ni and Zn in lakes on the Canadian Shield (Table 2-1). In contrast, measurements of labile metal in the water column are much more difficult to interpret in the context of metal bioavailability. For example, the DGT technique measures the rate at which a metal can diffuse through a protective gel and then react with an ion exchange resin, but diffusion in the water column towards a biological surface normally only very rarely limits metal uptake or toxicity (Degryse and Smolders 2016).

Table 2-1: Analytical techniques that yield estimates of the concentrations of the free metal ion or 'labile' metal.

Metal form	**Approach**	**Technique**	**Metal**	**Reference**
free metal ion	electrochemistry	ISE: ion-selective electrode	Cu	Luider et al. (2004); Rachou et al. (2007)
		AGNES: Absence of gradient Nernstian equilibrium stripping	Cd Pb Zn	Zavarise et al. (2010); Parat et al. (2015); Chen et al. (2018)
		CLE-CSV: competitive ligand equilibration - cathodic stripping voltammetry	Co Cu Ni Pb Zn	Monticelli and Caprara (2015)
	equilibrium ion-exchange	column ion exchange	Cd Cu Zn	Fortin et al. (2010)
		in situ ion exchange	Cd Co Ni Zn	Crémazy et al. (2015)
labile metal	electrochemistry	anodic stripping voltammetry	Cd Cu Pb Zn	Monticelli and Caprara (2015)
	diffusion gradient in thin films	diffusion gradient in thin films (DGT)	Cd Cu Ni Pb Zn	Degryse and Smolders (2016); Osterlund et al. (2016)

2.3 BLM conclusion

The BLM was already fairly mature by 2008 and the review in the MEND (2009) report is still an accurate and useful summary, particularly for the well-studied metals (Cd, Cu, Ni, Pb, Zn). To evaluate the bioavailability of these metals, in dissolved form (i.e., in the water that overlies submerged tailings and in the pore water residing in the tailings interstices), the BLM approach remains the obvious tool to use. In other terms, for laboratory exposures of aquatic organisms to cationic divalent metals and their hydrophilic complexes, in chemically defined media, there is good evidence that BLM yields reasonable predictions of metal uptake and toxicity. However, it must be admitted that results are far less numerous for experiments run under natural conditions, or in the laboratory in the presence of natural dissolved organic matter (DOM). This was the case when the 1993 and 2008 MEND reviews were written and it still prevails. Results from the few quantitative experiments that have been carried out under these conditions are inconclusive, being more-or-less evenly divided between examples that conform to the predictions of the BLM and others that appear to be in contradiction (see Table A-2 for a summary of these experiments). However, even in these cases, the BLM approach is demonstrably better than the use of the total dissolved metal concentration for assessing metal bioavailability (which was the approach prior to 1990).

A number of BLM models are freely available (Table 2-2). Of particular note are:

- the Bio-Met model (v.4: http://bio-met.net/), available from ARCHE, a Belgium-based consulting company; it can handle Cu, Ni, Pb and Zn;
- the Windward model (v. 3.1.2.37: http://www.windwardenv.com/biotic-ligand-model/#) that handles Al, Cd, Co, Cu, Ni, Pb and Zn.

In a good example of the application of the BLM to real-world metal situations, Smith et al. (2015) provide an overview of the BLM concept and identify challenges that have arisen when implementing the BLM at what they refer to as ‘mined and mineralized’ sites. One of the challenges cited by the authors is the need to deal with historically incomplete datasets for BLM input parameters, especially dissolved organic carbon (DOC). However, measurements of DOC are now fairly routine and for future applications to subaqueous disposal facilities this should not be a problem. Smith et al. (2015) also raise concerns about the nature of the DOC, referring in particular to DOC fractionation in Fe- and Al-rich systems and differences in DOC quality that result in variations in its metal-binding affinities. This may be a legitimate problem in low-pH and thus Fe- and Al-rich systems, but in a subaqueous disposal facility that is functioning properly (i.e., drainage with a near-neutral or basic pH), this fractionation should not be important. The authors also suggest that interpretation of BLM predictions should be tempered with considerations of other influences on metal bioavailability, such as multiple metal toxicity, toxicity attributable to the uptake of diet-borne metals, and increased tolerance to metal toxicity observed for aquatic organisms living in areas with elevated metal concentrations (see Section 1.2).

Table 2-2: Downloadable versions of the Biotic Ligand Model for different metals

Metal	**Reference**	**Comment**
Ag		
Cd	http://www.windwardenv.com/biotic-ligand-model/	Formerly HydroQual
Co	http://www.windwardenv.com/biotic-ligand-model/	Formerly HydroQual; acute and chronic toxicity
Cu	http://www.windwardenv.com/biotic-ligand-model/	Formerly HydroQual; acute toxicity
Ni	http://bio-met.net/	BIOMET, chronic toxicity
	https://www.wfduk.org/resources/rivers-lakes-metal-bioavailability-assessment-tool-m-bat	M-BAT, user-friendly version of BIOMET
	http://www.pnec-pro.com/	PNEC-pro
Pb	http://www.ila-lead.org/responsibility/lead-blm-tool	full BLM, chronic toxicity
	http://bio-met.net/	BIOMET, chronic toxicity, user-friendly (DOC, Ca, pH only)
	http://www.pnec-pro.com/	PNEC-pro
	http://www.windwardenv.com/biotic-ligand-model/	Formerly HydroQual; acute toxicity
Zn	http://www.windwardenv.com/biotic-ligand-model/	Formerly HydroQual; acute and chronic toxicity

3 Metal – Organism Interactions (ingested solids; food)

3.1 Biodynamic modelling

As described in the preceding section, the BLM is an equilibrium-based approach; it focuses on the interaction of a given metal with an external (epithelial) membrane, and it assumes that the epithelial membrane can be considered to be in equilibrium with the exposure medium (as shown in Figure 2-1). In contrast, the dynamic multi-pathway bioaccumulation model (DYM-BAM), an alternative way to consider metal-organism interactions, is based on a kinetic approach. Unlike the BLM, which only considers aqueous metal exposures, the DYM-BAM model explicitly takes into account metal uptake from both water and ingested solids (food), and it also considers metal elimination and growth dilution. At steady state, the bioaccumulated metal concentration can be predicted according to the eq. 3-1 (Luoma and Rainbow 2005):

$$\frac{d[\mathrm{M}]_{\mathrm{organism}}}{dt} = k_u\left[\mathrm{M}^{z+}\right] + (\mathrm{AE} \times \mathrm{IR} \times [\mathrm{M}]_{\mathrm{food}}) - k_e[\mathrm{M}]_{organism} - k_g[\mathrm{M}]_{\mathrm{organism}} \quad \text{(eq. 3-1)}$$

where k_u, k_e and k_g are rate constants for M-influx from water ($L^{-1}\cdot g^{-1}\cdot d^{-1}$), M-efflux ($d^{-1}$) and growth ($d^{-1}$), respectively, $[M^{z+}]$ is the free metal-ion concentration in water ($nmol\cdot L^{-1}$), $[M]_{organism}$ is the M concentration in the organism of interest ($nmol\cdot g^{-1}$ dry weight), $[M]_{food}$ is the metal concentration in the food consumed by the organism ($nmol\cdot g^{-1}$ dry weight), AE is the efficiency (%) with which the organism assimilates metal M from its food, and IR is the rate at which food is ingested by the organism (g prey$\cdot g^{-1}$ animal$\cdot d^{-1}$). In other words, the model integrates information from the animal's habitat (metal concentrations in water and food) and physiological data (influx rates from water, influx rates from food, rate constants of loss, and growth rates), to yield estimates of the steady-state metal concentration in the target organism.

As is clear from eq. 3-1, the DYM-BAM model requires detailed physiological information about the target organism. To obtain the required input data (e.g., food ingestion rates, the concentration-dependence of metal uptake from water and food, or the rate of metal elimination after ingestion), elaborate experiments must be run in the laboratory; in this regard, the data-requirements for the BLM are somewhat simpler.

Luoma and Rainbow (2005) reviewed the use of biodynamic modelling to predict species-specific metal bioaccumulation in the field. In a comparison of model predictions with field observations, for 6 metals and 1 oxyanion (Ag, Cd, Co, Cu, Cr, Zn; Se) and 14 animal species, whole-organism metal concentrations varied by over seven orders of magnitude. To accommodate this very wide range of body concentrations, the data were log-transformed and results for the same animal but from different studies were pooled. On a log-log scale, most predictions agreed with observations within a factor of two, with an overall coefficient of determination (r^2) between predictions and observations of 0.98.

In principle, one could use the dynamic modelling approach to estimate the internal dose of a given metal, and then relate this dose to a metal-induced effect (Sappington et al. 2011). However, experiments in the laboratory and in the field have shown that if the internal dose is reached slowly, the effects on the organism's function are reduced and its chances of survival are greater than if the internal dose is attained more rapidly. Stated differently, if the internalization of the metal occurs reasonably slowly, some degree of acclimation can be inferred (e.g., by turning on its metal-detoxification pathways and sequestering the metal in a non-available form, or by accelerating the metal efflux rate). On the other hand, if the internalization flux is high, the host animal is unable to detoxify all of the incoming metal, a critical internal dose is attained, function is reduced and the organism eventually succumbs (Andrès et al. 1999; Baudrimont et al. 1999). It follows that to use the DYM-BAM approach to predict metal toxicity (rather than metal bioaccumulation), one should consider not only the calculated steady-state metal concentration, but also the rate at which this internal dose is reached.

Note too that the biological parameters in DYM-BAM models are considered to be modelling 'constants', which are normally determined in the laboratory on metal-naïve test organisms. As discussed earlier in the BLM discussion (section 2.2.3 – *Acclimation*), recent research suggests that this assumption is likely too simplistic. Specifically, the key epithelial properties of aquatic organisms that govern metal accumulation and toxicity may well be affected by any antecedent exposure to the metal. Organisms that are chronically exposed to metals can alter the characteristics of their epithelial membranes and influence both the affinity of the metal-binding sites and the rate at which the metal is internalized (Niyogi et al. 2004).

Given these two factors (i.e., the need to consider metal influx rates and the differences between metal-naïve organisms and field exposed organisms), and despite its acceptance in a research environment, the DYM-BAM model has not yet been widely applied in a regulatory context.

3.2 Dietary uptake of metals

One of the inherent advantages of the DYM-BAM approach is that is considers the dietary uptake of metals. Until fairly recently, this route of metal uptake had been neglected (except for Hg and Se, where the importance of diet-borne metals was recognized early on (e.g., Adams et al. (2005)). An important catalyst for interest in diet-borne metals was a controversial publication by Fisher and Hook (2002), reporting that when marine copepods were exposed to metals (Ag, Cd, Hg, Se, Zn) through their diet, their reproductive capacity decreased by up to 75% (fewer eggs were produced and the hatching success declined for the eggs that were produced). These sublethal effects occurred at metal concentrations 2-3 orders of magnitude below dissolved metal concentrations that were acutely toxic, casting doubt on the validity of water quality criteria based on dissolved metals. The authors suggested that metals accumulated in copepods by trophic transfer would reach internal tissues, where only modest increases above background levels can depress reproductive capability. In their experiments, metals accumulated from the dissolved phase deposited primarily on external surfaces, where they had negligible biological effects at environmentally realistic concentrations. This distinction between the accumulation of waterborne and diet-borne metals is not surprising for Hg and Se, but is atypical for Ag, Cd and Zn and casts some doubt on the generality of the authors' conclusions.

Not coincidentally, the Society for Environmental Toxicology and Chemistry (SETAC) organized a workshop on the toxicity of diet-borne metals to aquatic biota in 2002. The resulting multi-authored book (Meyer et al. 2005) is a good introduction to the topic, with chapters dealing with a variety of subjects, including digestive physiology, toxic effects of diet-borne metals (in the laboratory and in the field) and the incorporation of diet-borne metals into regulatory frameworks.

Digestive physiology varies considerably among different animals, both in terms of the prevailing chemical conditions (e.g., pH, redox potential) and the length of time the food spends in the digestive tract (Campbell et al. 2005). For example, conditions in the digestive tract of zooplankton are not greatly different from those in the ambient water and the literature suggests that zooplankton only access the dissolved metals present within their prey. For deposit-feeding benthic invertebrates, most of the available information comes from the marine environment. Measurements of *in vivo* fluid properties (redox potential, pH, dissolved oxygen) in the guts of such deposit feeders suggest that the pH is close to neutrality and the oxygen concentration is vanishingly low (Weston et al. 2004). In contrast, digestion in higher animals is much more destructive and the passage time in their GI tract is longer than in zooplankton or small deposit feeders. Accordingly, the assimilation efficiency for the metals present in a prey item (‘AE’ in eq. 1) will vary from one prey item to another, as well as from one consumer to another. Negative effects of ingested metals may occur in the digestive tract itself (e.g., effects on the intestinal membrane or on the intestinal microbiome; Clearwater et al. (2005)), or the metals may enter the animal’s circulatory system after traversing the intestinal membrane barrier and thereby reach other internal organs.

Many studies probing the toxicity of diet-borne metals have been carried out since 2005, for different aquatic animals (particularly zooplankton and fish). Wang (2013a) reviewed progress in this field and qualified it as “slower than anticipated due to major obstacles such as uncertainly about the realism of toxicity testing using contaminated diets”. In the specific case of zooplankton feeding on metal-contaminated phytoplankton, Ag and Cd have displayed the greatest toxicity whereas the toxicity of Cu and Zn has been less obvious. In his review, Wang highlighted problems such as uncontrolled metal leakage from food items into the aqueous exposure medium, changes in the nutritional quality of the prey as induced by metal exposure and changes in the palatability of the prey as their metal concentration increased (resulting in a reduced feeding rate by the consumer). He concluded that “much remains to be learned about the mechanisms of dietary toxicity” and how these mechanisms differ from those induced by waterborne metal exposure.

In a subsequent and more extensive review of the toxicity of diet-borne metals, DeForest and Meyer (2015) considered a wide range of metals and several oxyanions (Ag, Al, Cd, Co, Cr, Cu, Ni, Pb, Zn; As, B, Mo, V). In the first phase of their review, they compiled a comprehensive database of toxicity data for diet-borne metals, with an emphasis on chronic toxicity, and in the second phase they addressed the question of whether water quality guidelines or criteria for metals based only on waterborne exposure are sufficiently protective. To quote from the review, “In general, adverse effects to organisms simultaneously exposed to waterborne and diet-borne metal were greater than when exposed to water or diet alone, but this was not always the case”. In particular, the authors looked for examples where diet-borne toxicity was observed in laboratory exposures when the diet-borne concentrations resulted from exposure of the food to waterborne concentrations near or below toxicity thresholds; such examples were identified for 6 of the target elements addressed in the present report (Ag, Cd, Cu, Ni, Zn; As). Based on their meta-analysis on the relevant literature, DeForest and Meyer identified some of the remaining uncertainties and made useful recommendations about the type of research needed to reduce those uncertainties.

3.3 Bioaccumulation of contaminants through the freshwater food chain

As is shown in the preceding sections, metals are subject to trophic transfer and can pass up aquatic food chains as a result of uptake from the diet. However, what is of particular interest is whether or not this trophic transfer results in increasing metal concentrations as one moves up the food chain, a phenomenon known as biomagnification and known to occur for methylated mercury. For organic contaminants, researchers tend to distinguish between *bioconcentration* (i.e., uptake from water) and *bioaccumulation* (i.e., simultaneous uptake from both water and food). However, this distinction is not rigorously respected by researchers in the metals sector, where it is more common to distinguish between ‘waterborne’ and ‘diet-borne’ metals.

Trophic Transfer Factors (TTF) are used to describe the relationship between the steady-state metal concentrations in an aquatic organism and the metal concentrations in its ambient environment (i.e., in water and food). They can be calculated for single or multiple transfers (steps) in a food chain, with each step representing a consumer-prey relationship. TTFs are analogous to bioaccumulation factors (BAFs) and can be used to determine if biomagnification is occurring (i.e., when “the TTF is > 1.0 through three or more trophic levels as a result of at least two trophic transfers” – Cardwell et al. (2013)). The latter authors reviewed published data on single and multiple trophic transfers for five metals, three that are biologically essential (Cu, Ni, Zn) and two non-essential elements (Cd, Pb). For laboratory experiments, they compared the observed TTFs with those calculated from a standard biodynamic model (eq. 3-1) by isolating the dietary component as follows:

$$TFF = \frac{\mathrm{AE} \times \mathrm{IR}}{k_e + k_g} \qquad \text{(eq. 3-2)}$$

where, as described earlier, AE is the efficiency (%) with which the organism assimilates metal M from its food, IR is the rate at which food is ingested by the organism (g prey·g^{-1} animal·d^{-1}), and k_e and k_g are rate constants for M-efflux (d^{-1}) and growth (d^{-1}). For the field studies, they examined studies for which the trophic levels had been assigned on the basis of stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$); $\delta^{15}N$ allows one to infer the relative position of a particular species in a given food web (Cabana and Rasmussen 1994), whereas $\delta^{13}C$ can be used to identify particular food sources, provided that these sources have different isotopic signatures (France 1995).

Not surprisingly, trophic transfer factors in a given food chain are metal-specific, and the TTF for a given metal varies from one food chain to another. Nevertheless, Cardwell et al. (2013) found reasonable agreement among trophic transfer factors calculated from laboratory experiments, field studies and biodynamic modelling. From their study, they concluded that Cd, Cu, Ni, Pb and Zn “generally do not biomagnify in (freshwater) food chains consisting of primary producers, macroinvertebrate consumers and fish"; in fact, the tendency is rather for 'biominification', with TTFs < 1. A similar conclusion was reached in the NRCC (1988) report on *Biologically Available Metals in Sediments*, where it was stated that "biominification – a reduction in metal levels at higher positions in the food chain – appears to occur frequently". Cardwell et al. (2013) did note some exceptions for Zn, in Zn-deficient situations where the dietary Zn concentrations were below those needed for metabolism in the consumer. For all the studied metals, there was an inverse relationship between the TTF and the actual concentration of the metal in the exposure medium, as has been reported for metal bioaccumulation factors. For example, McGeer et al. (2003) examined bioconcentration and bioaccumulation factors (BCF; BAF) that had been reported in the literature for Cd, Cu, Pb, Ni and Zn, and they demonstrated that there was a clear inverse relationship between BCF or BAF and aqueous exposure (see Figure 3-1 from their paper, below).

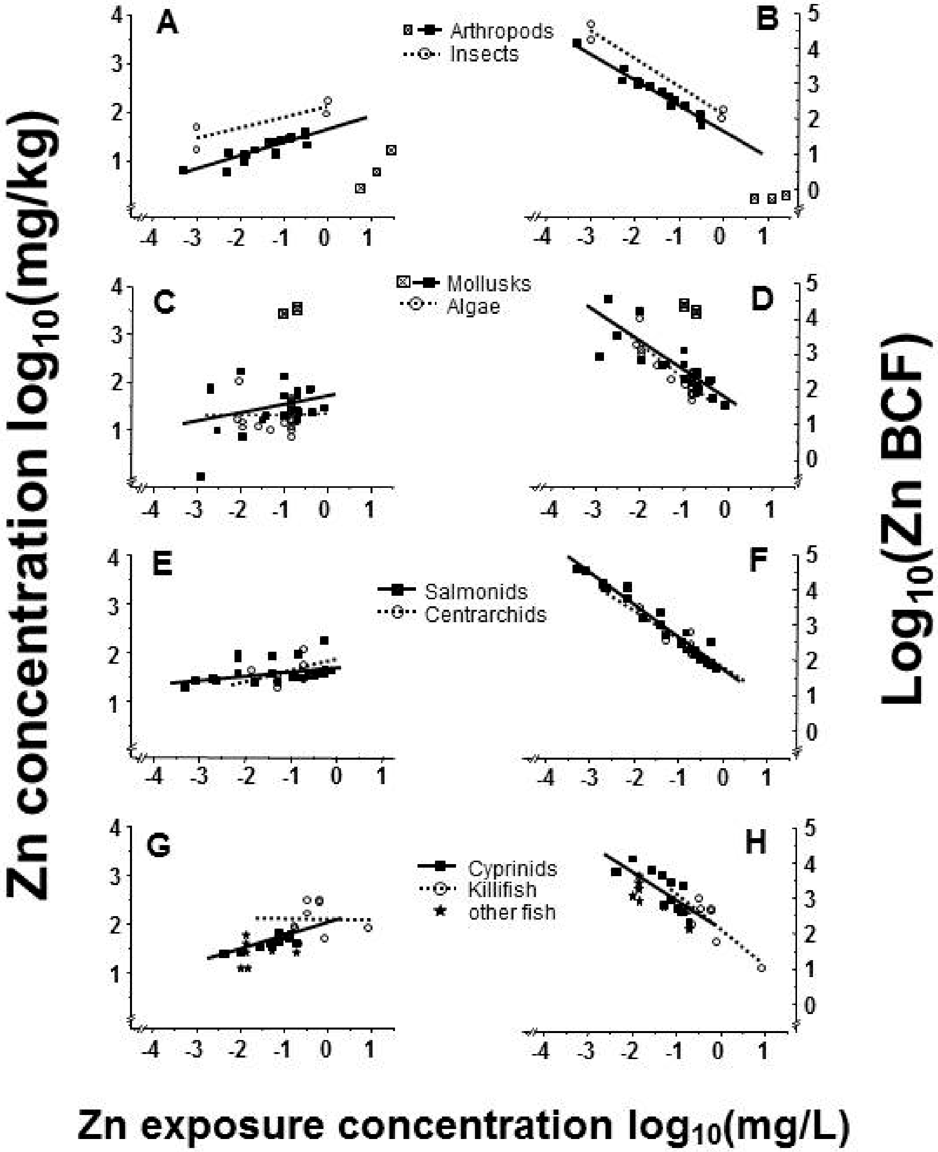

Figure 3-1: Effect of chronic Zn exposure on Zn content in aquatic biota (A, C, E, G) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Zn (B, D, F, H).

Data are on a log-log basis and the best fit line from the linear regression analysis is shown for each species group. Figure reproduced from McGeer et al. (2003), with permission from J. Wiley & Sons Publishers.

4 Metal Bioavailability in Sediments / Subaqueous Tailings

4.1 Introduction

The bioavailability of metals in (contaminated) sediments[6] has been extensively investigated over the past 30+ years. A useful starting point for the current update is the first MEND report in this series (MEND 1993), which drew heavily from an earlier NRCC report that focused on this topic (NRCC 1988). An abbreviated extract from the executive summary of the MEND (1993) report is cited below.

> Submerged mine tailings, and their constituent metals, may affect aquatic life in two ways: indirectly (through 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).
>
> To evaluate indirect exposure, one needs to estimate metal concentrations in sediment pore waters (i.e., $[M]_i$). Pore-water concentrations 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 $[M]_i$: one applies to oxic conditions and assigns control of $[M]_i$ to sorption reactions on such sorbants as Fe-, Mn-oxyhydroxides or sediment organic matter; the second applies to anoxic conditions and assumes that $[M]_i$ is controlled by precipitation-dissolution reactions with reactive amorphous sulphides (Acid-Volatile-Sulphides, 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 $[M]_i$ 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 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, the 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 constitutes 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

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[6] In the context of this report, 'contaminated' sediments are those where the metal levels are (well) above the local geological background concentration; such elevated concentrations may result from historical or ongoing anthropogenic activities or from natural surficial mineralization. There is no presumption that these concentrations pose an immediate or long-term hazard to the environment.

predict $[M]_i$ based on the geochemistry of the tailings after diagenesis and admixture of natural particulate material.

In the 20+ years since the MEND (1993) report appeared, there has been some oscillation between the two approaches outlined above (oxic *vs.* anoxic control of pore-water metal concentrations). Researchers at the Institut national de la Recherche scientifique (INRS) maintained an interest in the sorption equilibrium approach and demonstrated its use for predicting free metal ion concentrations in surficial sediment pore waters and using these calculated concentrations to predict steady-state metal concentrations in rooted aquatic plants and in freshwater bivalves (papers cited in MEND (2009) – see Appendix B). In parallel, researchers in the USA, notably at Manhattan College and within the US Environmental Protection Agency, introduced the SEM – AVS approach and tested its ability to predict the toxicity of sediment-bound metals (US EPA 2000). More recently, the pendulum has swung back towards a more nuanced vision of what controls metal cation concentrations in sediment pore water, with an appreciation that both sorption and precipitation reactions can intervene as the oxygenation of the surface sediment changes (e.g., seasonally or as a function of drought) and as the sediment ages (Costello et al. 2011; Nguyen et al. 2011; Vandegehuchte et al. 2013; Besser et al. 2015; Costello et al. 2016).

Since the aim of both approaches is to arrive at an estimate of the free metal ion concentration in sediment pore water, this being recognized as the best way to evaluate the bioavailability of a sediment-associated metal, the following section considers how to obtain $[M^{z+}]_i$, whether it be by calculation or by measurement. Since virtually all the examples will be drawn from the literature on contaminated sediments, in section 7 we consider how the differences between submerged tailings and natural sediments might affect the reasoning/recommendations.

4.2 Pore-water metal concentrations

Pore-water metal concentrations open a window into the geochemistry of sediment and submerged tailings, in that they reflect the chemical activity of metals present in the sediment matrix[7]. From an analytical standpoint, one needs a technique that provides an accurate and reproducible estimate of the dissolved metal concentration in the sediment interstitial water. Note that the pore-water approach assumes that, for sediment-ingesting animals, the physicochemical conditions in their digestive system are similar to those in the host sediment and that pore-water metal concentrations accurately reflect the chemical 'activity' of the ingested sediment-bound metal. Intuitively, based on our knowledge of digestive chemistry (see section 3.2 on digestive physiology (Campbell et al. 2005)), one might expect this assumption to be closer to reality for benthic deposit-feeding invertebrates than for benthic-feeding fish.

Traditionally, sediment pore water was obtained by coring the sediment, separating individual strata, and then extracting the pore water by filtration, centrifugation or squeezing. This approach has been largely replaced by the use of various passive sampling devices (Table 4-1). A critical review of these various devices was published in 2014 (Peijnenburg et al. 2014). Dialysis arrays or pore-water 'peepers'

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[7] Note that the same approach is used for organic contaminants – e.g., see Di Toro et al. (1989).

(Figure 4-1 (a-b)), consisting of diffusion cells inserted into the sediment and allowed to equilibrate, yield the least ambiguous results. They provide a time-averaged estimate of the ambient metal concentrations, which are particularly relevant in the case of long-term exposure of aquatic organisms that only slowly come to steady-state with respect to metal exposure in their environment. They also reduce/minimize the risks of sample contamination; for example, when deployed *in situ*, any contamination initially associated with a pore-water peeper or a diffusion cell will be nullified by dilution during the equilibration phase.

Care must be taken to ensure that the insertion of the peeper (or other sampling device) into the sediment does not disturb the sediment chemistry. For example, sediment chemistry can be readily altered by the introduction of oxygen into the sediment itself or into the pore-water sample held in the diffusion cells. To prevent this artifact, the peepers must be deoxygenated in a nitrogen atmosphere before deployment (Carignan et al. 1994). When the sampled zone includes both oxic and sub-oxic layers, the pore-water samples must be handled rapidly and preserved to maintain all the original solutes in solution; if acidification is used to preserve the samples, the pore-water pH must obviously be measured before the preservation step.

The pore water obtained after equilibration can be analyzed (total M, dissolved organic carbon (DOC), other cations and anions, pH) and the analytical results used as input data into a chemical equilibrium model (e.g., WHAM VII, Tipping et al. (2011); Visual MINTEQ, Gustafsson (2013)) to estimate the free metal ion concentrations. Note that sampling pore water directly with peepers is not metal-specific; it can be used for both cationic metals and for oxyanions (Table 4-1).

In addition to pore-water peepers, rhizons have also been used to collect interstitial water from sediments (Figure 4-1 (c)), usually from sediment cores into which the rhizons are inserted through ports in the walls of the coring tube. However, only relatively few analytes have been successfully analyzed (Fe, Mn, Si). The other techniques listed in Table 4-1 rely upon kinetic (flux) measurements and the quantitative link to biological availability is much more difficult to ascertain. For example, diffusion gradients in thin films (DGT) samplers give an estimate of 'labile' metals, labile being operationally defined by the nature and thickness of the gel through which the metals must diffuse and by the strength of the binding phase. In such cases, to obtain a concentration estimate, one must back-calculate the concentration that would have yielded the observed metal accumulation rate. This is relatively simple for synthetic solutions in the laboratory, but in field deployments the rate of metal accumulation can be affected by such factors as turbulence, temperature, water chemistry (pH, hardness, [DOM]) and by fouling of the device, and thus the back-calculation of the ambient concentration becomes much less certain (Peijnenburg et al. 2014; Osterlund et al. 2016).

4.3 Partial extractions of sediments

In cases where the sampling of the pore water directly is not feasible, metal concentrations in pore water can be estimated by determining the pH of the interstitial water and then subjecting the host sediments to partial extractions. The use of partial or sequential extractions to determine the partitioning of metals in freshwater sediments and their accumulation in the principal accumulative phases has a long and somewhat controversial history (Tessier et al. 1979; Rapin et al. 1986; Kheboian and Bauer 1987; Tessier and Campbell 1988; Nirel and Morel 1990; Tessier and Campbell 1991; Besser et al. 2015). The fractions obtained by such an approach are operationally defined, but provided that (i) the sediments are properly preserved before extraction (Rapin et al. 1986) and (ii) the extraction procedure is matched to the redox status of the sediment (Huerta-Diaz et al. 1993), the results can be interpreted with some confidence. A common error found in the literature is the application of extraction procedures designed to be used on oxic sediments (e.g., the Tessier et al. (1979) and Community Bureau of Reference or BCR (Quevauviller et al. 1993) protocols) to anoxic sulphide-containing sediments. Similarly, extraction procedures that were developed for fresh (wet) sediments have been applied to air- or oven-dried sediments, without consideration of the changes in metal partitioning that are likely to occur as the sediment pore water is removed.

For *oxic* sediments, partial extractions are normally chosen to extract specific metal-binding phases in the sediments (e.g., Fe or Mn oxyhydroxides) and to quantify both the concentration of the binding phase and the concentration of the associated metals. This approach was described in detail in the first MEND report (1993) and successful applications of the approach were updated in the 2009 report. The main assumptions behind the approach are summarized in Table 4-2 and a compilation of successful applications can be found in Appendix B (Table B-1).

The approach described in Table 4-2 is basically a simplification of a full surface complexation model or SCM. SCMs for metal binding to hydrous iron oxides (Dzombak and Morel 1990) and hydrous manganese oxides (Tonkin et al. 2004) have been incorporated into the TICKET-Unit World Model (Farley et al. 2011), which is structured to consider the fate of a metal in a lake (including its sediments). This model has been updated since its original publication and the current version now allows one to simultaneously consider acid-base reactions, metal complexation in solution, metal precipitation (e.g., metal hydroxides, carbonates, sulphides) and metal binding to Fe-oxides, Mn-oxides and organic matter (Kevin Farley, personal communication, 2017-11-13). When these phases are present together in sediments, they are assumed to react in the same manner as they do when present singly in the laboratory. The results reported by Peña et al. (2011) for the sorption of Ni on biomass-birnessite assemblages support this assumption (bacterial biomass and birnessite, a layered manganese oxide harbouring both Mn(III) and Mn(IV)): “Our results thus support the view that the biofilm does not block reactive mineral surface sites; instead, the organic material contributes to metal sorption once high-affinity sites on the mineral are saturated.”

For *anoxic* sediments, an extraction with dilute HCl is often used to dissolve the amorphous sulphide phases (AVS) and the associated metals (SEM or simultaneously extracted metals; Ag, Cd, Cu, Ni, Pb, Zn) (Allen et al. 1993). Total SEM (ΣSEM) is calculated as the sum of concentrations of reactive metals in sediment:

$$\sum SEM = \frac{1}{2}SEM_{Ag} + SEM_{Cd} + SEM_{Cu} + SEM_{Ni} + SEM_{Pb} + SEM_{Zn} \text{ (eq. 4-1)}$$

The molar difference between ΣSEM and [AVS] has been shown to be a good predictor of the presence or absence of sediment metal toxicity.[8] According to the original formulation of the SEM – AVS model, if the concentration of AVS exceeded ΣSEM, the sediment metals were assumed to have reacted with the sulphide to become insoluble, nontoxic metal sulphides, whereas if [AVS] was less than ΣSEM, some of the metals were predicted to remain in solution (Hansen et al. 1996) and were therefore considered to be bioavailable. This simplistic formula did not account for metal partitioning to other sediment solid phases such as particulate organic matter, and it could only predict the absence of toxicity, not the onset of toxicity induced by sediment metals. Di Toro et al. (2005) refined this approach and used a sediment biotic ligand model, based on an equilibrium partitioning model of sediment toxicity (Ankley et al. 1996) and the Windemere Humic Aqueous Model (Tipping 1994) to predict acute and chronic toxicity values for sediment metal concentrations, accounting for both AVS and partitioning to organic carbon. The median lethal concentration (LC_{50}) of excess SEM normalized by sediment organic carbon ($SEM^*_{x,OC}$) can be calculated using the equation 4-2:

$$SEM^*_{x,OC} = (SEM^* - AVS)/f_{OC} \quad \text{(eq. 4-2)}$$

where f_{OC} is the fraction of the organic carbon concentration in the sediment. Somewhat surprisingly, Di Toro et al. (2005) reported that the $SEM^*_{x,OC}$ values for Cd, Cu, Ni, Pb and Zn remained essentially unchanged despite differences in pore-water chemistry, including hardness, salinity, and dissolved organic carbon, with the exception of pH. They suggested that an SEM*x,OC threshold value of >100 µmol/g organic carbon could be used to predict when metal toxicity might be expected to occur in the sediments (see Figure 4-2). Basically, Di Toro et al. belatedly realized that if the SEM – AVS value were positive, that didn't necessarily mean that all the excess metal was 'bioavailable'. Rather, it might be bound to other sediment solid phases, such as organic matter or, given the micro-heterogeneity of sediments (micro-niches), it might be bound to iron oxyhydroxides.

It has been suggested that one of the reasons for the relative success of the SEM – AVS approach in laboratory and field studies is that the 1 M HCl extraction of the sediments is simultaneously extracting both the SEM and some of the metals weakly bound to Fe/Mn hydrous oxides and organic matter. In their original papers on the use of chemical extractions to predict the bioavailability of metals in oxic (estuarine) sediments, Jenne and Luoma (1977) used a dilute HCl extractant (0.1 N HCl, much weaker than for AVS determinations). Hydrochloric acid is an unselective extractant and protons will tend to desorb at least some of the specifically adsorbed metals (including those held on the Fe hydrous oxide surface). The HCl extraction would also dissolve carbonate and moderately soluble sulphate minerals. In other words, although the SEM – AVS method was based on anoxic sediment principles, it will also remove some metals from oxic sediments. This observation could partially explain why SEM/AVS ratios must reach 2 or higher before benthic effects are observed. Although the occurrence of SEM/AVS benthic effect ratios >2 has been attributed to metal binding to organic matter (Mahony et al. 1996), metal binding to Fe and Mn hydrous oxides may also be playing an important role.

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[8] Note that the molar difference between ΣSEM and [AVS] is not a good predictor of metal bioaccumulation, i.e., it should not be used to predict how much metal will be taken up by a test organism or (in the field) how much will be measured in benthic organisms collected along a SEM – AVS gradient.

4.4 Diagenesis (post-depositional changes in sediments over time)

4.4.1 Diagenesis in freshwater sediments

Knowing that sediments are the major biogeochemical sink for metals and oxyanions and that they potentially constitute a useful environmental archive, paleolimnologists have traditionally collected sediment cores, sectioned them into individual sediment strata and analysed each stratum for its metal content. By dating the cores (e.g., ^{210}Pb, with validation from other chronological markers), they have then interpreted the vertical metal profiles in the sediment core as a record of historical metal deposition.

To use cores in this fashion, one must assume that when a metal-bearing particle reaches the sediment-water interface, the metal remains immobile and is slowly buried as additional particles settle to the lake bottom. Given that the sediment-water interface is known to be a biologically diverse and very active zone, both from a microbiological point of view (settled organic particles are subject to biodegradation) and from a benthic invertebrate perspective (feeding and burrowing behaviour in surficial oxic sediments), it is not necessarily true that the foregoing condition is met for all metals (Carignan et al. 2003).

Indeed, whole books have been written about the 'early diagenesis' of sediments, a term that encompasses the physical, chemical and biological processes that affect a sediment particle after it has been deposited from a water body (Berner 1980; Boudreau 1997):

- physical processes = diffusion, advection, compaction;
- chemical processes = pH changes, oxidation-reduction, precipitation-dissolution, adsorption-desorption, complexation-decomplexation;
- biological processes = uptake-elimination, bioturbation, bioirrigation.

In the present context, we will first consider how diagenesis affects metal mobility in a lake or pond sediment, and then address the much less voluminous literature on submerged mine tailings.

As implied in the preceding paragraph, metal inputs to sediments are usually dominated by the sedimentation of autochthonous and allochthonous particles.[9] However, in contaminated lakes, metals can diffuse from the water column into the sediments, provided that dissolved metal concentrations above the sediment-water interface are higher than in the sediment pore water (Cu, Ni: Carignan and Nriagu (1985); Zn: Carignan and Tessier (1985)). Such conditions are more likely to occur in acidic lakes than for sediments overlain by circumneutral waters.

Diagenesis models usually treat sediments in a single (vertical) dimension and they take into account most if not all of the following processes (Berner 1980; Boudreau 1997):

- sedimentation of particles from the overlying water;
- compaction of the settled sediment;

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[9] Autochthonous particles are those generated within the water body, for example by algal photosynthesis, breakdown of aquatic vegetation, or *in situ* mineral precipitation, whereas allochthonous particles are those that originate from the catchment or from atmospheric deposition.

- diffusion of oxygen (and other solutes, including metals) from the overlying water into the sediment;
- biodegradation of organic matter;
- reduction of a sequence of electron acceptors (O_2, NO_3, Mn, Fe, SO_4, CO_2) – Markelova et al. (2017);
- diffusion of the dissolved products of these reactions and their possible reactions (adsorption, precipitation);
- bioturbation (particle mixing by benthic animals);
- bioirrigation or biodiffusion (movement of pore water, provoked for example by organisms pumping overlying water through their burrows – Gallon et al. (2008)).

Labile organic matter and microorganisms are the key drivers for diagenetic changes; the microbial communities involved are strongly influenced by the sediment redox conditions (and they, in turn, play a key role in determining the redox state of the sediment). Given the importance of oxygen as the initial electron acceptor, it is particularly relevant to consider the diffusion of O_2 across the sediment-water interface and into the sediment. The flux of O_2 will be affected by the porosity and tortuosity of the sediment. Porosity, defined as the ratio of the volume of interconnected water in a sediment divided by the total sediment volume, is inversely related to grain size (Berner (1980), p.27). Sediments with small particles (e.g., appreciable clay content) are subject to slight compaction as a result of their own accumulating weight, even over short distances (cm), with the result that the water content of clay-rich sediments can be higher in the upper sediment layer than in lower layers. Tortuosity results from the presence of particles, in that the O_2 molecule (or indeed any solute) is not free to diffuse in any direction, as it would be in the overlying water, but instead must negotiate a tortuous path in the interstitial water, avoiding the sediment particles; tortuosity can be expressed as eq. 4-3

$$\theta = dl/dx \quad \text{(eq. 4-3)}$$

where dl is the length of the actual path taken by the solute over a depth interval of dx; since $dl > dx$, $\theta > 1$. The diffusion coefficient in a sediment matrix, D_s, can be expressed in terms of tortuosity as $D_s = D/\theta^2$ (Berner 1980), a relation demonstrating that, as expected, the diffusion coefficient in a sediment will be lower than that in an aqueous solution, D. However, the calculation of the tortuosity of a real sediment, e.g., on the basis of its porosity, is difficult (Berner 1980); several models have been proposed to relate tortuosity to porosity in porous media with particles of different sizes and shapes, and although the details of the models differ (Ghanbarian et al. 2013), they all agree in predicting that tortuosity will decrease with an increase in porosity. However, two different sediments with similar porosity but differing in particle size, particle aggregation or organic content may well exhibit different tortuosities.

Actual measurements of O_2 concentrations in sediment pore water, using O_2-sensitive microelectrodes, bear this out; oxygen can penetrate up to 10 cm depths in sandy sediments, but in silts the oxic-anoxic interface is rarely deeper than 1 cm (e.g., Davison et al. (1991), 1 mm; Berg et al. (1998), 25 mm; Bryant et al. (2010), 1-2 mm; Couture et al. (2016), 8-30 mm). Note that the depth of penetration of O_2 into sediment will also depend on the rate at which it is consumed, which in turn will be affected by the amount and composition (lability) of the organic matter present in the sediment. Measurements on submerged tailings (Vigneault et al. 2001) and on biofilms overlying tailings lying on the bed of intermittent streams (Haack and Warren 2003) showed that O_2 penetrated to less than 10 mm (Vigneault et al. (2001), 8 mm; Haack and Warren (2003), 2 mm).

4.4.2 Post-depositional changes in sediments

Research addressing the question posed in the previous section, regarding the interpretation of depth profiles of metals in lake sediments and whether they truly reflect the chronology of metal inputs to the lake catchment, has been carried out over the last 15+ years by Canadian researchers, focusing on lakes on the Canadian Precambrian Shield and to a lesser extent on lakes in the Appalachian range in southern Quebec (Richard Carignan, Université de Montréal; André Tessier and Charles Gobeil, INRS). Their approach has involved the careful collection of paired sediment cores and sediment pore water. The lakes were chosen on the basis of their distance from metal smelters and their airborne emissions, the otherwise undisturbed nature of their watersheds, and their low watershed to lake surface area ratios (to maximize the influence of atmospheric deposition and minimize watershed influences). In some cases the deep waters were perennially oxic whereas in others a seasonally anoxic hypolimnion developed that minimized bioturbation and bioirrigation effects.

The pore-water profiles were fitted to a one-dimensional transport-reaction equation and analytical data from the paired pore-water and solid phase profiles, assuming steady state. Inverse modelling of the profiles allows one to identify depth intervals (zones) where the metal is either released to or removed from pore water and to quantify net reactions rates in each zone. The metals studied have included Cd (Alfaro-De la Torre and Tessier 2002), Cu (Carignan et al. 2003), Pb (Gallon et al. 2004), Re (Chappaz et al. 2008b), Tl (Laforte et al. 2005), U (Chappaz et al. 2010), In (Tessier et al. 2014), Zn (Perron 2005), As (Couture et al. 2008; Couture et al. 2010) and Mo (Chappaz et al. 2008a).

For several metals and oxyanions (e.g., Pb; As, Mo), there was evidence for mobilization in the zone where reductive dissolution of iron oxyhydroxides was occurring, and for removal lower down in the sediment profile by precipitation as a solid sulphide. However, the overall influence of these diagenetic reactions on the metal concentration profiles in the sediment cores was negligible for most of the studied metals, and thus post-depositional mobility of the metal did not exert a significant effect on the observed solid-phase metal profiles in the sediment.

Cd: “The shape of vertical profile of total Cd concentration with depth in the sediment appears to be determined more by its input history than by post-depositional mobilization and redistribution in the sediment column” – Alfaro-De la Torre and Tessier (2002)

Cu: “… some dissolved Cu is lost from the upper 2 cm of the sediment to the overlying water. Subtracting the diffusive loss from the total Cu profile provides an estimate of the Cu deposition history at this site, corrected for diagenesis. In this particular case, the correction amounts to about 10% only” – Carignan et al. (2003)

Pb: “… diagenetic processes, such as remobilization, molecular diffusion, bioturbation, and bioirrigation have a negligible influence on the solid phase Pb profile” – Gallon et al. (2004)

U: “diagenetic reactions involving U are not quantitatively important and … most of the U was delivered to the sediments at our study sites as particulate U and not through diffusion across the sediment-water interface” – Chappaz et al. (2010)

Zn: Profiles of dissolved Zn across the sediment-water interface were consistent with diffusion of Zn from the water column into the sediment, and loss of Zn at depth by precipitation as ZnS or reaction with FeS. However, this flux of Zn into the sediment had a negligible effect on total particulate Zn in 3 of the 4 lakes studied, and only a slight effect (≤ 5%) in the fourth lake – Perron (2005)

Exceptions to this generalization include thallium, rhenium and arsenic, for which the post-depositional remobilization and transport either to deeper (anoxic) layers or to the overlying water altered the metal concentration profile in the sediment core.

Tl: Contrasting behaviour of Tl was reported for two lakes, one that was perennially oxic and the other with a seasonally anoxic hypolimnion. In the perennially oxic lake, the pore-water Tl concentration profiles displayed a peak below the sediment-water interface, suggesting post-depositional Tl remobilization and transport to the overlying water and to deeper sediments. In the lake with a seasonally anoxic hypolimnion, Tl diffused downward across the sediment-water interface and was immobilized by co-precipitation with and/or adsorption to Fe sulphides. In this latter case, "The concentrations of Tl removed from or added to sediments after their deposition (i.e., diagenetic Tl) amount to up to 36% of measured sediment Tl concentrations" – Laforte et al. (2005)

Re: In four lakes, one permanently oxic and three with seasonally anoxic bottom waters, Re concentrations in the water overlying the sediment were consistently higher than in the sediment pore water. Rhenium diffused across the sediment-water interface and its concentrations in the sediment pore water decreased with depth to reach minimum values (0.5 pM) within a 10-cm sediment depth interval, the decrease being attributed to precipitation of ReS_2. Accordingly, "accumulation of sedimentary Re shows a strong authigenic component" – Chappaz et al. (2008b)

As: The behaviour of As was studied in two basins in the same lake, one perennially oxic and the other seasonally anoxic (Couture et al. 2008; Couture et al. 2010). In the former case, total sediment As concentrations increased sharply upward close to the sediment surface due to scavenging of upward diffusing As by Fe oxyhydroxides. In contrast, As concentrations in the seasonally anoxic basin decreased towards the sediment surface where As scavenging by Fe oxyhydroxides did not occur. Further work in the perennially oxic basin, using a one-dimensional reactive transport model describing the coupled biogeochemical cycling of As, C, O, Fe, and S, demonstrated that the depth profile of sediment-bound As reflected both the past changes in As deposition and the diagenetic redistribution of As among the Fe(III) oxyhydroxide and Fe(II) sulphide pools**.** Using the model, it could be shown that organic matter degradation in the sediment and atmospheric sulfate deposition to the lake were influential factors affecting the benthic recycling of As. Lower organic matter degradation resulted in more oxidizing conditions and a greater abundance of Fe(III) oxyhydroxides in the topmost sediment, which act as a reactive barrier to diffusion of As in the sediment pore water (cf. Martin and Pedersen (2002), Martin and Pedersen (2004)). Variations in sulphate availability were predicted to have complex effects on benthic As remobilization, since sulphide produced by sulphate reduction could enhance both the removal of dissolved As through precipitation of Fe(II) sulphides (i.e., co-precipitation and/or adsorption of As with Fe monosulphides) and the release of dissolved As through the reductive dissolution of Fe(III) oxyhydroxides.

4.4.3 Post-depositional changes in submerged tailings

In moving from sediments in metal-contaminated lakes to submerged tailings in a subaqueous disposal facility (SAD), there are a several differences to consider. Submerged tailings from massive sulphide ore bodies will be very rich in reduced sulphur and normally poorer in organic carbon than typical lake sediments. Recall that in lake sediments organic carbon and its degradation by heterotrophic microorganisms is the main driver for maintaining reducing conditions below a thin oxic layer at the sediment-water interface. Note, however, that this suggested difference in organic carbon content would not apply if the water cover were shallow and the submerged tailings were host to rooted aquatic vegetation. Although post-glacial lakes are naturally heterogeneous , one might also expect a greater heterogeneity in a SAD facility than in a lake, given that the tailings introduced into the facility are likely to vary over time (i.e., over mine life) and vary over space due to differential settling and resultant winnowing as the tailings spread out from the inflow spigot. Tailings may also contain process chemicals used during the ore beneficiation such as flotation agents.

Relatively few studies of post-depositional changes in submerged tailings have been published in the peer-reviewed literature. In an early study, Pedersen et al. (1993) compared the chemistry of pore waters and the associated solid phases in tailings and organic-rich natural sediments collected in Anderson Lake, Manitoba, a small, shallow and highly productive lake (≥ 0.6 m water cover; pH 6.8-7.4). Sampling was carried out in 1990, 11 years after the mine started disposing of its tailings in the lake. However, the lake is not a clear-cut example of a SAD disposal, since at the time of the sampling it was receiving not only tailings and process water, but also surface water metal inputs due to acid rock drainage in the catchment. Three cores were collected (one in pure tailings, one in a section of the lake where no tailings had been discharged, and a third where tailings were overlying the original lake sediments). Pore water was collected by sectioning the cores under nitrogen, centrifuging, and collecting the supernatant water. At all sites, the deposits were anoxic within several millimetres of the sediment-water interface, as evidenced by high concentrations of dissolved Fe(II). The concentration gradients between the overlying water and the pore water demonstrated that both the tailings and the natural sediment were acting as a sink for metals, not a source (cf. section 4.5.1). Based on the dissolved sulphate profiles across the tailings-water interface, and on the observation of framboidal pyrite in the surface layers, Pedersen et al. (1993) suggested that the metals entering the tailings or sediments were being precipitated as autogenic sulphides. They concluded that tailings stored under a permanent water cover showed “a lack of post-depositional (diagenetic) chemical reactivity”. In a parallel study on Buttle Lake on Vancouver Island in British Columbia, Pedersen (1983) reached a similar conclusion, that no significant oxidation of the submerged tailings was occurring and that there was no release of Cd, Cu or Zn from the tailings into the overlying water.

A similar study was carried out by Martin et al. (2001) in a Peruvian lake (Lago Junin) affected by mining activities (Cu, Pb and Zn sulphide deposit). This lake had been influenced by the activities of upstream mining operations, which contributed acid rock drainage (ARD), tailings and tailings pond overflow to a river that drains into the lake. In this regard, the nature of the inputs was not typical of a properly constructed and operated subaqueous disposal facility. As was the case in Anderson Lake, the deposits in the main lake basin became anoxic within a few millimetres of the water-sediment interface, and dissolved metal concentration profiles across this interface indicated that the S-rich deposits (2.5 weight percent) in the main basin were acting as a sink for Cu, Pb and Zn. In contrast, the periodically exposed deposits in the shallower parts of the lake showed strong evidence of sulphide oxidation (pore-water pH values < 4) and release of Cu and Zn to the overlying water. This contrast between the permanently submerged deposits and those that were alternately unsaturated and submerged nicely illustrates the importance of maintaining a permanent water cover; periodic wetting and drying episodes tend to accelerate the sulphide oxidation processes.

In another study, carried out on a somewhat less confusing site, Martin et al. (2003a) examined the diagenetic behaviour of sulphide-rich mine tailings that had been placed in a subaqueous disposal facility at the Quirke mine, near Elliot Lake in northern Ontario. Note that the tailings had been subject to subaerial oxidation before their submersion. Pore-water profiles of dissolved oxygen, Fe and Mn were determined 20 years after mining operations had ceased. In the shallow areas (< 1.5 m water depth), characterized by sparse filamentous vegetation, well-mixed water columns and fully oxygenated bottom waters, these profiles indicated that sub-oxic conditions prevailed below depths of 3 cm in the tailings. Minor remobilization of Ni, As, Mo and U was noted in these shallow areas but the release of these elements to the overlying water was limited by scavenging in the interfacial oxic horizons. In the deeper zones of the facility (> 2 m depth), the presence of aquatic vegetation (*Chara* sp.) fostered stagnant bottom waters and favoured the development of anoxic conditions above the tailings-water interface. These anoxic tailings were characterized by substantial remobilization of ^{226}Ra (linked to a depletion of a mixed (Ra,Ba)SO_4 phase) and minor remobilization of dissolved As, Ni and Zn. Martin et al. (2003) suggested that the removal of trace metals in the anoxic bottom waters was limited by the availability of free sulphide.

Also in eastern Canada, Vigneault et al. (2001) carried out a two-year study on the fate on sulphidic mine tailings stored under a shallow water cover in replicate experimental cells constructed at the site of the Louvicourt Mine, near Val d'Or , Quebec.[10] In this experimental setup, the 'confounding factors' mentioned earlier, such as metal inputs from ARD in the adjacent catchment, were eliminated and the water cover was permanent. Oxygen microelectrodes were used to determine O_2 profiles across the tailings-water interface, and as was the case in the two previous studies, there was minimal penetration of O_2 into the tailings (<57 mm). Elevated concentrations of H_2S, Fe(II) and Mn(II) were measured below the interface, indicating persistent anoxic conditions at depth. The very shallow water cover (0.3 m) favoured light penetration and photosynthesis, and a biofilm developed at the tailings-water interface. Unlike the two lake studies, which suggested that submerged tailings were effectively inert, in this study there was some evidence of oxidation in the uppermost layer (depletion of dissolved O_2 at the surface, pH↓, [SO_4]↑). The presence of sub-surface peaks in the concentrations of Cd and Zn in the tailings pore water suggested some mobilization of these two metals from this surface layer, followed by their flux from the tailings into the overlying water. The indication that the tailings were acting as a source of

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[10] For a more complete description of the Louvicourt Mine tailings facility, which was adjacent to the experimental cells, see Ouellet et al. (2011). No studies were carried out within the tailings impoundment itself, but this report does present data for pH and metal concentrations in the effluent from the facility, which demonstrate the effectiveness of the water cover in preventing acid generation.

metals, rather than as a sink, contrasts markedly with the results discussed above for Anderson Lake, Buttle Lake and Lago Junin. In these three cases, the lakes were receiving appreciable inputs from their respective catchments and the dissolved metal concentrations were markedly higher than in the Louvicourt Mine experimental cells.

In a follow-up study, Vigneault et al. (2007) revisited the Louvicourt experimental cells in 2005, 9 years after the initial submersion of the tailings and 7 years after the earlier measurements. Based on the mineralogical and chemical data, the authors confirmed that mobilization of trace metals had occurred in surface tailings but also concluded that that the overlying biofilm was effectively trapping the released metals. The Cd and Zn fluxes to the overlying water had decreased 10-fold between 1998 and 2005, and for Cu the submerged tailings had switched from a sink to a source of Cu for the water cover. Vertical microprofiles of pH and oxygen across the tailings-water interface and biological analyses of the biofilm community structure indicated that the biofilm acted both as a source and a sink of H^+-ions and oxygen, depending on diurnal light conditions. Vigneault et al. (2007) concluded that biofilm development was beneficial since it offered protection against wind-induced resuspension of the tailings and contributed to the maintenance of anoxic conditions in the underlying sediments. In addition, the biofilm trapped mobilized dissolved metals and decreased fluxes out of the tailings into the overlying water column. Note, however, that a subsequent study by Kwong et al. failed to find extensive biofilm development in the Louvicourt Mine tailings disposal facility itself or at four other sites (MEND 2010). They concluded that "the formation of microbial mats over submerged tailings does not appear to be a common phenomenon".

As a complement to these studies, Moncur et al. (2015) recently published a study of tailings from the Sherritt-Gordon Cu-Zn mine in Sherridon, Manitoba. Over the life of the mine, most of the tailings were deposited in tailings impoundments, but at the end of mining in 1951 some tailings were slurried and deposited into Fox Lake. Cores were collected in 2001 and 2009, at two locations – one was an area with a permanent water cover of about 1 m, whereas the other area was exposed to the atmosphere. The exposed tailings did not support vegetation, but much of the submerged tailings were overlain by a layer of naturally established vegetation and detritus. Low-resolution pore-water concentration profiles were obtained from the cores by extrusion followed by squeezing of individual strata (25 cm) (Moncur et al. 2013); in addition, piezometers were inserted into the submerged tailings at different depths below the tailings-water interface to extract greater volumes of pore water (12, 38, 63 and 88 cm below the tailings-water interface). Both sites consisted of well-sorted fine to medium-grained sand, suggesting that they had been located close to a tailings discharge point.

Mineralogical analyses and X-ray absorption near edge structure (XANES) spectroscopy confirmed that alteration of the sulphide minerals within the submerged tailings was confined to a thin zone (< 6 cm) located immediately below the water-tailings interface, whereas in the sub-aerial tailings the zone of alteration extended to 60 cm (the water-table depth). The composition of the pore water in the subaqueous tailings (circumneutral pH, mildly reducing redox values, moderate alkalinity, relatively low concentrations of dissolved sulphate and metals) was consistent with the absence of significant alteration of the sulphide minerals. Interestingly, there was evidence of dissimilatory sulphate reduction (DSR) including elevated populations of sulphate reducing bacteria (SRB), elevated pore-water H_2S

concentrations, and strong $\delta^{34}S-SO_4$ and $\delta^{13}C$-dissolved inorganic carbon fractionation. The likely source of organic carbon for the SRB was the overlying vegetation and the dissolved organic carbon in the surface waters.[11] Overall, these findings, obtained some 60+ years after mine closure, are consistent with the earlier results from the studies were carried out on recently deposited tailings. Moncur et al. (2015) concluded that “the use of subaqueous tailings deposition as an approach to limiting oxidation may be effective over extended time periods”.

4.5 Biomonitoring

In this section, the term ‘biomonitoring’ has been interpreted broadly, to include (i) chemical analysis of aquatic organisms to determine their metal content as a means of assessing the bioavailability of the metals present in their environment (sections *4.5.1* and *4.5.2*), (ii) transcriptomic analysis of the biomonitor organisms to assess whether their metabolism has been perturbed by exposure to the metals (section *4.5.3*), and (iii) taxonomic analysis of the aquatic communities, to determine whether these communities have been affected by metal exposure (section *4.5.4*).

4.5.1 Total metal bioaccumulation

A direct way to evaluate the bioavailability of metals in sediments is to collect native biomonitor organisms from the site (see section 3.4.2 in MEND (1993)). This can be done by sampling the indigenous fauna and flora at the site (‘passive’ biomonitoring) or by transplanting metal-naïve plants or animals into the water body and allowing them to interact with the sediments (‘active’ biomonitoring). In both approaches, there is an obvious need to have nearby reference sites that can be used for comparison purposes.

The biomonitor species can be chosen to target either the sediments or the overlying water. For example, some rooted aquatic plants have been shown to obtain nutrients (e.g., phosphate – see Carignan and Kalff (1980)) and some metals (e.g., As, Cd, Cu, Zn – see Wolterbeek and van der Meer (2002)) from the sediment. In the latter experiment, the metals taken up by the roots tended to stay there, with minimal translocation to the shoots. However, research on other species has shown that root to shoot translocation can be important (e.g., Cu, Pb – see Denny (1980); Welsh and Denny (1980)). In addition, direct uptake of dissolved metals by foliage has also been reported for some aquatic plants (Fritioff and Greger 2007). A similar situation exists for benthic invertebrates, some of which have been shown to take up most of their metal via sediment ingestion (e.g., tubificid larvae) whereas many others live in the sediment but create their own micro-environments (burrows) and derive their metals burdens not from the sediment but from the overlying water that they use to irrigate their burrows (Warren et al. 1998). In other words, the choice of biomonitor plant or animal should be guided by what is known about the relative importance of the sediment or overlying water as a metal source (cf. Sections 3.1 and 3.1).

An obvious advantage of the biomonitoring approach is that the bioaccumulated metal concentrations provide an estimate of the overall metal bioavailability in a given water body (integrating waterborne

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[11] In addition to contributing organic matter to submerged tailings, rooted aquatic plants may also act as sources of O_2 through root radial oxygen loss (Jacob and Otte, 2004), which would favour sulphide oxidation.

and diet-borne metals). In a risk assessment context, there are fewer uncertainties when metal concentrations in a biomonitor species are measured, rather than extrapolated from aqueous metal concentrations or sediment metal concentrations – use of the organism itself obviates the need to estimate the 'bioavailability' of the metal in the water or in the ingested food.

Measures of bioaccumulated metals have an additional potential advantage in that they can be used within the framework of a Tissue Residue Approach for toxicity assessment. The science behind using whole-body or tissue residues as a dose metric for characterizing toxic responses and for setting environmental quality guidelines or criteria was reviewed at a SETAC Pellston workshop held in 2007; the results of the workshop were published in one of the SETAC journals (*Integrated Environmental Assessment and Management (IEAM)* **7** (1) (2011)) and among the six papers, several deal peripherally with metals and organometals (McElroy et al. 2011; Sappington et al. 2011) and one paper considers metals exclusively (Adams et al. 2011). The latter authors noted that species-specific and site-specific approaches have been developed for several metals (case studies are presented for Cd, Ni and Se), but that "tissue residue assessment for metals other than organometals has not led to the development of a generalized approach as in the case of organic substances". As is described in the following section (*4.5.2*), aquatic organisms tend to accumulate metals in different forms, some of which remain internally bioavailable whereas others are detoxified; measures of total bioaccumulated metal ignore this question of subcellular metal partitioning. If the ratio of bioavailable to detoxified metal remains constant with increasing exposure, then the tissue residue approach is appropriate. However, if the subcellular partitioning of the metal varies with metal exposure, then the tissue residue approach no longer applies. Note too that the whole-body or tissue residue concentration above which toxicity occurs will be organism-specific – a common critical metal threshold concentration does not exist.

Criteria that are commonly applied in the selection of appropriate biomonitor species are listed in Table 4-3 (Phillips and Rainbow 1993; Luoma and Rainbow 2008a), and examples of freshwater biomonitor organisms for metals can be found in Table 4-4. Typically, a researcher would look for biomonitor species that have demonstrated a wide range of metal concentrations when collected along a metal contamination gradient. In submerged tailings or contaminated lacustrine sediments, it would make sense to select biomonitors that have a clear link to the benthic environment, either because they live in direct contact with the sediments themselves or because other benthic species are an important component of their diet. In some cases such as small animals (e.g., insect larvae or zooplankton), the whole organism can be analysed, but in the case of larger animals (e.g., bivalve molluscs or benthic-feeding fish) it is possible and often more informative to dissect individual organs and analyse them separately (e.g., livers or digestive glands; gills).

Within a given species and a site, bioaccumulated metal concentrations often vary as a function of age or size (Luoma and Rainbow 2008b). A common approach is to collect specimens within a narrow age or size range at every site; however, as an alternative, it is also possible to collect (at each site) as wide a range of size as possible and then plot bioaccumulated metal concentrations as a function of size at each site and compare the curves obtained at each site. Such plots can also be used to calculate and compare the metal concentrations for a 'standard size' organism for each sample site.

One of the recognized limits of the biomonitoring approach is that it may not work well for essential metals that are under tight homeostatic control[12] (unless, as in the case of barnacles (Rainbow and White 1989), this control involves long-term storage of the metal). For example, bioaccumulated Cu and especially Zn concentrations in freshwater animals tend to be relatively insensitive to changes in the ambient metal concentrations (Hare and Tessier 1998; Giguère et al. 2004). Note too that for some biomonitor organisms, their gut content may bias the measurements of 'bioaccumulated' metal; this is particularly important for small sediment-ingesting benthic organisms (Hare et al. 1989), for which the metal content of the ingested sediment may exceed the true bioaccumulated metal content. For such biomonitors, it is important to allow them sufficient time to clear their gut and to provide conditions favourable for gut clearance (e.g., provide them with clean food).

4.5.2 Subcellular metal partitioning

As a refinement to the approach described in the preceding section that deals with the total bioaccumulated metal concentration, one could also consider the subcellular fate of the metal. In effect, the bioaccumulated metals may be detoxified, for example by sequestration in insoluble granules, by isolation in membrane-bound vesicles, or by complexation with cytosolic ligands (Mason and Jenkins 1995). If this is the case, the plant or animal harbouring the metal will normally not be affected by the accumulated metal (except perhaps indirectly, if there is an appreciable metabolic cost to the detoxification). The form in which the bioaccumulated metal exists may also affect its fate if the plant or animal is consumed as food (Wallace et al. 2003; Wang and Rainbow 2006; Rainbow et al. 2007). For example, metals that have been detoxified and are present as insoluble metal-rich granules tend to pass right through the digestive system of the consumer without being assimilated, whereas metals present in soluble forms in the cytosol are normally more available for assimilation and available to exert a toxic effect (Meyer et al. 2005).

Several techniques have been proposed to differentiate between detoxified metals and biologically available metals (Wallace et al. 2003; Wallace and Luoma 2003). In such techniques, one must first isolate the different metal-binding ligands and then determine the amount of metal associated with each ligand pool. Methods currently used to perform the isolation step tend to fall into one of two classes, differential centrifugation or high performance liquid chromatography (HPLC) or some combination of the two. In all cases, the biological tissue of interest must first be homogenized in a cold, isotonic medium as gently as possible, to avoid disrupting membrane-bound cellular organelles (e.g., nuclei, mitochondria, microsomes, lysosomes) (Rosabal et al. 2014a). Once the sample has been homogenized, differential centrifugation can be used to separate the various organelles (Rainbow 2002; Wallace et al. 2003; Wallace and Luoma 2003) as a function of their settling velocities (Figure 4-3). To complete the differential centrifugation approach and to determine the speciation of the dissolved cytosolic metals, the supernatant remaining after the final ultracentrifugation (Figure 4-3: supernatant S4) can be heated, cooled and subjected to a second ultracentrifugation step. The heat-denaturable proteins will be found

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[12] In this context, homeostatic control refers to the ability of living organisms to maintain the concentrations of certain key solutes in a narrow range, for example by increasing uptake when the intracellular concentrations drop too low and by increasing efflux when the intracellular concentrations rise above a certain threshold.

in the pellet, whereas heat-stable proteins and peptides such as metallothionein will remain in the supernatant.

Alternatively, the heating and cooling step can be skipped and liquid chromatography (LC) used to separate the different cytosolic molecules involved in metal binding. A variety of chromatographic approaches have been adopted to perform this separation. Size-exclusion chromatography (SEC) is often used as the first step (Mason et al. 1990) followed in some cases by ion-exchange chromatography (Mason and Meraz 1998) or by capillary electrophoresis (Prange et al. 2001). Lobinski and Szpunar (1999) provide a useful summary of various 'hyphenated' techniques that can be used to separate and quantify metal complexes with macromolecules.

The operational fractions identified in Figure 4-3 have been grouped into different categories: '*BAM* or *biologically active metals*' (heat-denatured proteins or HDP + mitochondria + microsomes), '*BIM* or *biologically inactive metals*' (metal-rich granules + heat-stable proteins and peptides or HSP) and '*TAM* or *trophically available metals*' (cytosolic metals + organelles) (Wallace et al. 2003; Rainbow et al. 2007). This approach makes sense intuitively, but most published studies have focused on determining whether or not the subcellular partitioning of a metal changes as the total metal concentration increases (e.g., in lakes representing a metal concentration gradient). Only a few papers have convincingly demonstrated a link between changes in subcellular partitioning and the onset of toxicity (see Campbell and Hare (2009) and Wang (2013b) for reviews).

In freshwater examples published since 2009, Eyckmans et al. (2012) exposed rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and gibel carp (*Carassius auratus gibelio*) to high but environmentally relevant Cu concentrations in the laboratory for up to 1 month. The three fish species are known to differ in their sensitivity to Cu exposure (sensitivity increases in the order gibel carp < common carp < rainbow trout). After exposure, gills and liver of control and exposed fish were homogenized and subjected to a differential centrifugation procedure. At the subcellular level, in the most sensitive species (rainbow trout) the additional Cu was found exclusively in the biologically active metal pool (BAM) whereas the most tolerant species (gibel carp) accumulated most of the excess Cu in the biologically inactive fraction (BIM); the behaviour of the common carp was intermediate, consistent with the order of sensitivity to Cu. Similar trends were observed in the liver. The authors concluded that "differences in Cu distribution at the subcellular level of gills and liver of these fish species strongly reflects their capacity to handle Cu excess and is one of the greatest contributors to their differences in sensitivity to Cu".

Cooper et al. (2013) subjected a freshwater unionid bivalve, *Pyganodon grandis*, to an abrupt step-change in metal exposure. Adult bivalves were transferred from a reference site (Lake Opasatica) and a metal-contaminated lake (Lake Héva) to a second contaminated lake (Lake Vaudray) in northwestern Quebec. Changes in gill and hepatopancreas metal concentrations (Cd, Cu, Zn), in the subcellular distribution of metals and in metallothionein concentrations were followed over time (t = 0, 132, 400 and 860 days). Metal detoxification strategies differed between target organs (in the gills, incoming metals were sequestered largely in Ca-rich granules, whereas in the digestive gland the same metals primarily accumulated in the cytosol, in the metallothionein-like protein fraction), but not among the three lakes. The strategies employed by the metal-naïve bivalves, originating in the reference lake, were similar to those identified in free-living *P. grandis* chronically exposed in the metal-contaminated lake; the authors concluded that the ability to handle incoming metals (Cd in particular) is inherent in *P. grandis*, is not a trait acquired after long-term adaptation of the bivalve to metal-contaminated environments, and that the overall distribution between BAM and BIM pools does not change as a function of the ambient metal concentration.

More recently, Jakob et al. (2017) examined two closely related amphipod species from Lake Baikal (Siberia), *Eulimnogammarus cyaneus* and *E. verrucosus*, which differ in body size (10- to 50-fold lower fresh weights of *E. cyaneus*) and sensitivity to waterborne Cd exposure. The smaller species exhibited higher Cd uptake rates and higher sensitivity for Cd (2.3-fold lower LC_{50} values). When exposed to species-specific sub-acute Cd concentrations (i.e., nominally LC_{01} values: 2.0 $\mu g \cdot L^{-1}$ and 12.9 $\mu g \cdot L^{-1}$ for the sensitive and tolerant species, respectively) for 4 weeks at 6 ^{o}C, Cd concentrations in the BAM fraction were similar for the two species but Cd concentrations in the BIM pool were 35% higher in the more tolerant species. In other words, the relative importance of the BAM and BIM pools was different in the two species. Interpretation of this comparison is difficult, however, since body size and the exposure concentrations were different for the two species.

4.5.3 Transcriptomic analysis

Parameters that have been used to detect metal exposure and effects in aquatic organisms include tissue metal concentrations, concentrations of metallothionein[13] (MT) and indicators of overall organism health, such as condition index, growth or reproductive endpoints. However, metal stress may induce subtle effects at the subcellular level that do not necessarily translate into changes in these more traditionally-measured variables, but that may still involve a detrimental metabolic cost.

Ecotoxicogenomics refers to the application of genomics to the field of ecotoxicology (Snape et al. 2004; Ankley et al. 2006), a trend that started in about 2000. The focus of this section is on 'transcriptomics' and the study of the 'transcriptome' of native organisms chronically exposed to metals in their environment. The term transcriptome is sometimes used to describe all the ribonucleic acid (RNA) molecules in a cell or a population of cells, but more often it is limited to the messenger RNA (mRNA). The cell genome, barring mutations, is constant in a particular organism, whereas the transcriptome reflects the genes that are being expressed and thus will tend to vary as function of the ambient conditions and the organism's physiological state. In this context, the transcriptome can be considered as an addition to the family of metal-sensitive 'biomarkers' that existed prior to 2000 (e.g., see Mussali-Galante et al. (2013)).

Transcriptomic investigations allow for the rapid assessment of the impacts of stressors on numerous biochemical pathways at the level of gene expression (mRNA). Since gene expression varies as a function of toxicant exposure, disease state and cellular metabolism, it offers a window into how cells, organisms and populations respond to changes in the external environment, whether these changes are natural or anthropogenic. The use of transcriptomic methods in ecotoxicology has the potential to yield a

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[13] Metallothioneins are cysteine-rich proteins involved in maintaining sufficient intracellular supplies of some essential metals (Cu, Zn) and in detoxifying excess intracellular metals (including non-essential metals, e.g., Cd).

mechanistic understanding of toxic effects on organisms or populations, and thus help to bridge the gap between cause and effect (Brinke and Buchinger 2016). In addition, because this approach allows the researcher to monitor the expression level of up to several thousand genes, belonging to many different metabolic pathways, it has the potential to generate chemical-specific signatures of toxicity; for organisms that are exposed to an array of potential stressors in their normal habitat, the availability of such chemical-specific signatures could in principle be used to determine which of the potential stressors are in fact affecting the indigenous biota (Denslow et al. 2007; Bougas et al. 2013).

Initially microarrays were the most popular method used for studying changes in mRNA expression, but Next Generation Sequencing (NGS) technologies such as RNA-seq are becoming the preferred method for studying a transcriptome. Using the RNA-seq, the following steps are involved in determining the transcriptome of a particular organism (list adapted from Denslow and Sabo-Attwood (2015) and Qian et al. (2014)):

- Extract or adsorb total RNA from cells or tissues of control and exposed organisms.
- Process the total RNA to select mRNAs.
- Prepare a double-stranded cDNA library, by RNA fragmentation prior to the reverse transcription or by reverse transcription first followed by cDNA fragmentation; fragmentation is needed to obtain the appropriate length for NGS technologies.
- Bind fluorescent sequencing adapters to both ends of the fragments.
- Amplify each fragmented cDNA molecule in an *in vitro* cloning step.
- Sequence the fragments using NGS technologies.
- Bioinformatic analysis of the resulting 'reads'.

With reference to this last step, a key resource for transcriptomic studies is the gene ontology (GO) database, which categorizes annotated genes into known biochemical pathways. The database uses a standardized vocabulary that includes biological process, molecular function, and cellular component. These GO terms can help in the identification of the biochemical pathways that are affected by toxicant exposure (Denslow et al. 2007).

Despite the obvious potential of transcriptomics, the application of this tool in ecotoxicology was initially hampered by the absence of the necessary genomic data for non-model species. Initial studies were restricted to short-term laboratory or transplantation experiments with model organisms for which large genomic resources had previously been developed (e.g., fathead minnow, rainbow trout). A major shortcoming of such short-term experiments was that they necessarily focused on changes in gene expression that resulted from a step-change in the exposure conditions. In such experiments, the organism suffers an initial shock and then initiates a series of responses designed to compensate for the effect of the toxicant; if the exposure duration is extended, without causing mortality, the organism will eventually reach a new steady-state in which gene expression stabilizes, representing a trade-off between the cost of detoxification and cost of allowing some low level of toxicity to persist so that some of the residual available energy can be used for growth and reproduction. This latter chronic exposure scenario is more relevant for a subaqueous disposal facility than would be a short-term exposure or a sudden change in exposure conditions.

With the introduction of NGS technologies, beginning in about 2008, the 'model organism' restriction was lifted and the transcriptomic approach could be applied to 'non-model' aquatic species. Using this approach, laboratory-reared animals can be exposed to water or sediment obtained from the field and changes in their transcriptome can be interpreted as a response to bioavailable contaminants present in the field sample. In a variant of this bioassay approach, laboratory-raised test organisms (e.g., fathead minnows or amphipods) can be caged in the field, at reference sites and at sites that are subject to contaminant inputs; differences in their transcriptomes can then be examined. Denslow and Sabo-Attwood (2015) recently reviewed such applications, and although the exposure of the caged animals was of short duration (48 h), they were able to conclude that "similar changes in gene expression patterns and pathways are found in multiple studies with the same toxicants, indicating that the responses are fundamental to the mode of action of the chemicals in aquatic organisms". A more realistic approach is to collect wild animals from their home environment, where they are chronically exposed to a contamination gradient, and to determine how their transcriptome varies in relation to contaminant exposure. Some examples of this type of application can be found in Table 4-5.

Contrasting with the positive assessment of field experiments by Denslow and Sabo-Attwood (2015), and with the earlier enthusiasm of Mussali-Galante et al. (2013) who suggested that 'omics' technologies were "reinventing the field of toxicology, providing 'molecular signatures' of exposure, enabling a more robust risk assessment than has ever been achieved previously", Bahamonde et al. (2016) provide a more sobering perspective. Using the Canadian Environmental Effects Monitoring (EEM) programme as an example of an established programme, the authors describe the steps that would be required for 'omics' technologies to be integrated into such a framework. They consider the whole range of 'omics' technologies, i.e., metabolomics, proteomics and transcriptomics; with respect to the latter approach, they correctly point out that natural variability in gene expression (seasonal, temporal, inter-organism) has not yet been quantified and that the impact of variables such as nutritional and reproductive status has not yet been constrained. In a similar vein, Brinke and Buchinger (2016) note that changes at the transcriptomic level can only be identified as 'effects' (in the EEM sense) if they are clearly linked to a specific stressor-induced effect at the population level such as decreased survival or lower reproductive capacity.

4.5.4 Taxonomy (e-DNA)

The use of taxonomic analysis of aquatic communities to assess the biological status of a stream or standing water body is a well-established but labour-intensive tool. It was considered in Section 5-2 of the MEND (1993) report ('*Effects of metals at the population and community levels*') and a summary of the main conclusions of that section can be found in Table 4-6 of the present update. In the development of the Canadian guidelines for the Environmental Effects Monitoring programme, as designed to address potential effects caused by metal mining effluents, attention was focused on fish population surveys to assess fish health, and a benthic invertebrate community survey to assess effects on fish habitat. In the 2012 updated guidelines, fish population survey endpoints include fish condition, relative liver weight, relative gonad weight, weight at age and age; benthic community survey endpoints include total density, taxon richness, the Bray-Curtis index of dissimilarity and Simpson's evenness. Details can be found in Chapters 3 and 4 of the Metal Mining Technical Guidance for Environmental Effects Monitoring (Environment Canada 2012).

The intent of the present section is not to update the taxonomic literature, but rather to introduce a new tool (environmental DNA or 'e-DNA'). With recent advances in techniques that permit the rapid and reasonably inexpensive sequencing of DNA, it has become possible to evaluate the diversity of species present in a given water body on the basis of a genomic analysis of samples collected with due care from the aquatic system of interest. Most published papers that deal with e-DNA tend to assume that the reader has a reasonable grasp of DNA sequencing, but fortunately Shaw et al. (2016b) have recently published what they have called 'A beginner's guide'. This guide provides a helpful introduction to this rapidly developing field and identifies the requirements that must be met before the technique can be effectively incorporated into a biomonitoring programme.

The term e-DNA refers to the DNA that is present in an environmental sample (water, sediment, soil, feces) and that can be sequenced and used to identify the taxa from which the DNA originated (e.g., by excretion, shedding skin, releasing gametes). Two different methods can be used to detect organisms present in a water body: (i) targeted sequencing, which focuses on a specific informative gene region, and (ii) shotgun sequencing, where all DNA fragments within a sample are sequenced regardless of their origin. In the context of a biomonitoring programme, a multispecies variant of the first approach known as 'metabarcoding' has been tested to obtain information about the biodiversity of an environment. With this approach, which involves the targeting of two evolutionally conserved DNA sequences separated by a highly variable species-specific sequence, groups of organisms such as plants, bacteria, algae, fungi or vertebrates can be detected. The steps involved in an e-DNA biodiversity study include (i) sample collection in sterile containers, (ii) DNA concentration and extraction, (iii) polymerase chain reaction (PCR) amplification of the target gene region with tagged primers, (iv) sequencing of the e-DNA on a next-generation sequencing (NGS) platform and (v) bioinformatic analysis. For details concerning these steps, recommendations concerning the available choices and the precautions to be taken, the reader is referred to the publication by Shaw et al. (2016b).

In a second paper, Shaw et al. (2016a) compared fish community data obtained either by e-DNA metabarcoding or by traditional netting in two complex river systems in a south-central Australia (the North Para and Murray Rivers). Using e-DNA, they detected 100% of the species caught in the fyke nets, including threatened and invasive species, when appropriate sampling strategies were used (five 1-L water samples enabled the detection of the less abundant taxa, whereas these taxa were not detected with two 1-L samples). In a comparison between sampling the water column or the sediments, the water column samples proved to be more effective for detecting fish communities than did the sediment samples. However, the authors also identified several issues that would have to be remedied if e-DNA were to be applied on a routine basis. These issues included the identification of a number of 'spurious species', presumably due to genetic similarities between closely related species (only one of which is known to exist in Australian fresh waters), or the introduction of e-DNA by transport vectors from other locations (currents from upstream, boats, predator feces, wetland birds). The authors identified the need to develop a local reference sequence database, and they recommended the use of multiple genetic markers. Finally, Shaw et al. (2016a) examined the relationship between sequence abundance and taxa abundance and found a positive relationship for some species, but given the many methodological factors that can influence the sequence count, they concluded that 'more research is needed before species abundance can be reliably estimated from eDNA metabarcoding surveys.' They also note that e-

DNA metabarcoding ‘cannot currently provide demographic information, such as sex, maturity, age, and abundance’ but they express optimism that markers to do so may be developed in the future.

The use of e-DNA to carry out monitoring surveys of benthic invertebrates has been considered by Carew et al. (2017), again in an Australian context. As was the case for fish, they too identify a need for DNA barcodes for local freshwater invertebrate species. They note that there is increasing pressure to push identification of benthos to the species level, to provide potential diagnostic information (since closely related species may differ in their sensitivity to a particular stressor – see Baird and Hajibabaei (2012)), but identification by traditional means is time-consuming, costly and requires specialized taxonomic knowledge and robust taxonomic keys (there are more than 10^5 species of freshwater invertebrates overall – Balian et al. (2008)). Carew et al. (2017) suggest that when done properly, e-DNA metabarcoding detects about 90% of the macroinvertebrate species present, but they reiterate that currently metabarcoding is best suited for species detection rather than for determining species abundance.

A Canadian scientist, Paul Hebert at the University of Guelph, was the first to propose biological identification through DNA barcodes (Hebert et al. 2003). Perhaps not coincidentally, a current University of Guelph researcher, Robert Hanner, is currently working with Mary Murdoch of Stantec Consulting Ltd. on the application of DNA to identify benthic invertebrates. The study was presented at the 2017 Canadian Ecotoxicity Workshop, in Guelph (Murdoch et al. 2017), and objectives as listed in the CEW abstract book were to determine the ability to recover DNA barcodes from formalin-fixed morphological voucher specimens collected from an EEM monitoring programme conducted at a mine site in Ontario (Alamos Gold Corporation), to test the ability of the existing Biodiversity Institute of Ontario’s barcode reference sequence library to provide matches to the sequences derived from the study specimens, and to compare the agreement between specimen identifications derived from morphology and those obtained from the barcoding approach. The need for proper preservation techniques was also discussed recently by Yamanaka et al. (2017), who examined the efficacy of various cationic surfactants as preservatives that could simply be added to e-DNA water samples to suppress its degradation. Finally, Furlan and Gleeson (2017) suggest some quality control measures, involving positive controls, that are designed to identify false negatives arising during the amplification stage only. Overall, the e-DNA approach is very promising but it clearly is not yet a mature technique.

Table 4-1: Sampling devices for metals in sediment interstitial water. [14]

• Dialysis arrays (pore-water peepers) (Al, As, Cd, Co, Cu, Hg, Fe, Mn, Ni, Pb, Zn) See Fig. 4.1(a, b)	Multi-layer diffusion cells inserted into sediments and allowed to equilibrate with the sediment pore water. Equilibration times can range from about 1 day to > 1 month, depending upon whether the resupply of analytes from the sediment solid phase is able to sustain the pore-water concentrations. The equilibration process has been modelled. Typical deployments of about 1-2 weeks are used.	Hesslein (1976); Carignan et al. (1985); Brandl and Hanselmann (1991); Nipper et al. (2003); Teasdale et al. (2003)
• Rhizons (Fe, Mn, Si) See Fig. 4.1(c)	Originally designed as micro-tensiometers to sample seepage water in the unsaturated zone in soils. Consist of hydrophilic porous polymer tube (typical pore diameter 0.1 µm), connected to a syringe or a vacuum tube by a PVC tube. Supporting nylon wire fixed to one end of the porous polymer. Fluid sampled from the sediment flows into the space between the porous tube and the supporting wire. Vertical resolution ≈1 cm. Usually used to extract IW from cores.	Seeberg-Elverfeldt et al. (2005); Dickens et al. (2007); Shotbolt (2010); Poigner et al. (2013)
• Teflon sheets (Fe, Mn + associated metals)	Teflon sheets act as a substrate for the deposition of iron and manganese oxyhydroxides and their associated metals; useful for identifying the boundary between the oxic and sub-oxic zones and adsorption of metals to new phases.	Belzile et al. (1989); Fortin et al. (1993); Feyte et al. (2010)
• Diffusive Gradients in Thin Films (DGT) samplers (As, Cd, Cu, Co, Hg, Mo, Ni, Pb, Sb, Se, V, Zn)	DGT deployments within sediments are influenced by sediment response to depletion of analytes in the pore waters. The mobilization of metals from sediment solid phase can lead to pore-water concentrations being fully or partially sustained. The response of sediment to DGT measurements has been characterized by several models, including interpretation of micro-niche responses. Metal pore-water distributions can be determined at high-resolution and in two dimensions.	Zhang et al. (1995); Davison et al. (1997); Davison et al. (2000); Martin (2008); Bennett et al. (2010); Stahl et al. (2012); Panther et al. (2013); Arsic et al. (2018)
• Diffusive equilibration in a thin film (DET) samplers (Cd, Cr, Cu, Fe, Mn, Mo, Pb, Re, U, Zn)	An equilibrium passive sampler that uses a thin layer of hydrogel as the sampling media, i.e., similar to DGT except that samplers do not have a binding phase and simply equilibrate with the pore water. Deployed in unconstrained or constrained modes, with the former requiring fixing to minimize diffusional relaxation and the latter using compartments. Can be used to make measurements of two-dimensional pore-water distributions at high-resolution.	Davison and Zhang (1994); Krom et al. (1994); Yu et al. (2000); Fones et al. (2001); Bennett et al. (2012)

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[14] Table adapted from Peijnenburg et al. (2014).

Table 4-2: Summary of the equilibrium adsorption (surface complexation) model. [15]

- Under oxic conditions, cationic metal concentrations in sediment pore water are controlled by <u>adsorption</u> reactions.
- Sorbents in oxic sediments = Fe oxyhydroxides, Mn oxyhydroxides, sedimentary organic matter.
- Individual sorbants present in the surface sediments compete for trace metals (analogous to the competition among dissolved ligands for the dissolved trace metals).

$$M^{z+} + \equiv S_1 - OH_x \xleftrightarrow{{}^*K_1} \equiv S_1 - OM + nH^+ \qquad (1)$$

$$M^{z+} + \equiv S_2 - OH_x \xleftrightarrow{{}^*K_2} \equiv S_2 - OM + nH^+ \qquad (2)$$

$$M^{z+} + \equiv S_3 - OH_x \xleftrightarrow{{}^*K_3} \equiv S_3 - OM + nH^+ \qquad (3)$$

$$\ldots. \quad + \quad \ldots. \quad \xleftrightarrow{{}^*K} \quad \ldots..$$

$${}^*\mathrm{K}_\mathrm{i} = \frac{<\{\equiv \mathrm{S}_i - OM\}>\left[H^+\right]^n}{<\{\equiv S_i - OH_n\}>\left[M^{z+}\right]} \qquad (4)$$

where S = sorbant (e.g., ‘S_1’= Fe(III) oxyhydroxide; ‘S_2’ = Mn(IV) oxyhydroxide; ‘S_3’= sedimentary organic matter); $<\equiv S_i\text{-}OH_n>$ = concentration of free binding sites on sorbant ‘i’ ; $<S_i\text{-}OM>$ = 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 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.

- Partial chemical extractions can be used to extract a particular sorbent (e.g., amorphous Fe(III) oxyhydroxides) and its associated metals, yielding eq. (5)

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

- <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 eq. (5), i.e. $[H^+]$ and $[M^{z+}]$, can be obtained by sampling and analyzing the pore water in equilibrium with oxic surface sediments.
- Using field measurements of this type, conditional (pH dependent) constants can be determined for the sorption of Cd, Cu, Ni, Pb and Zn on natural amorphous iron oxyhydroxides (Tessier 1992), and then used to calculate the free metal ion concentration in the pore water in a given sediment.

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[15] Extracted from MEND (1993), Section 2.1, and also described in Campbell and Tessier (1996).

Table 4-3: Criteria for choosing appropriate freshwater biomonitor species for metals.

Criterion	**Explanation**
associated with sediments	(none needed!)
limited home range (sedentary)	bioaccumulated metal can be related to a specific location
widespread (cosmopolitan)	ease of comparison of the same biomonitor species among different sites
abundant (not a threatened species)	sample collection will not affect species abundance
metal-tolerant	biomonitor species must be present in metal-rich environments or survive when introduced there
tolerant of manipulation	when absent from a contaminated site, can be transplanted into and maintained (caged) there
net metal accumulator	will yield a wide usable range of metal concentrations across different sites
known biology, known biodynamic constants	useful for calculating the length of time necessary to reach steady-state (active biomonitoring)
ease of collection and identification	reduces the need for taxonomic expertise
organism size	need sufficient tissue for multiple analyses

Table 4-4: Examples of freshwater biomonitor organisms for metals.

Species	**Metals**	**References**
Benthic invertebrates		
Pyganodon grandis (bivalve)	Cd, Cu, Ni, Pb, Zn	Couillard et al. (1993); Tessier et al. (1993); Bonneris et al. (2005); Perceval et al. (2006); Masson et al. (2010); Cooper et al. (2013)
Bithynia tentaculata; *Physa gyrina* (gastropods)	Cu, Zn; Cu, Ni, Pb	Flessas et al. (2000)
Chaoborus sp. (insect larva)	Cd, Ni, Se	Hare and Tessier (1998); Ponton and Hare (2009); Ponton and Hare (2013); Rosabal et al. (2014b)
Hexagenia limbata (insect larva)	Cd, Hg	Warren et al. (1998); Naimo et al. (2000); Michaud et al. (2005); Masson et al. (2010)
Hyalella sp. (amphipod)	As, Cu, Mn, Ni, Sb, Se, Tl, V, Zn	Shuhaimi-Othman et al. (2006); Borgmann et al. (2007); Couillard et al. (2008)
Benthic feeding fish		
Perca flavescens (yellow perch)	Cd, Cu, Ni	Giguère et al. (2004); Kraemer et al. (2008); Couture et al. (2015)
Catostomus commersonni (white sucker)	Cd, Cu, Hg, Se	Harrison and Klaverkamp (1990); Miller et al. (1992); Janz et al. (2014)
Rooted aquatic plants		
Nuphar variegatum	Cu, Zn	Campbell et al. (1985); Sprenger and McIntosh (1989); Pip and Stepaniuk (1992)
Vallinsneria americana	Cd, Cu, Ni, Pb, Zn; Fe, Mn	St-Cyr and Campbell (1994); St-Cyr and Campbell (1996); St-Cyr and Campbell (2000)
Periphyton	Al, Cd, Cu, Mn, Ni, Pb, Zn	Bailey and Stokes (1985); Stokes et al. (1985); Holding et al. (2003)

Table 4-5: Examples of applications of the transcriptomic approach to freshwater aquatic animals.

Species	Approach	Known contaminants	Reference
(a) laboratory-raised animals exposed to field-collected water			
Fathead minnow *Pimephales promelas*	laboratory fish, caged in the field (48 h); microarray	endocrine disruptors	Denslow and Sabo-Attwood (2015)
Fathead minnow *Pimephales promelas*	laboratory fish, exposed in the laboratory (48 h); RNA-seq	oil sands process water	Wiseman et al. (2013)
Rainbow trout *Oncorhynchus mykiss*	laboratory fish (juvenile), caged in the field (14 d), up- and down-stream from outfall; microarray	municipal wastewater effluent	Ings et al. (2011)
(b) wild-caught animals chronically exposed to metals and other contaminants in field situations			
Yellow perch *Perca flavescens*	wild fish, caught along metal contamination gradient; RNA-seq; liver	metals (Cd, Cu, Zn)	Pierron et al. (2011)
	wild fish from reference lake, transferred to and caged in contaminated lake (4 wks.); microarray; liver	metals (Cd, Cu, Zn)	Defo et al. (2015); Bougas et al. (2016)
	wild fish caught in St. Lawrence River, up- and down-stream from Montreal; microarray; liver	municipal and industrial effluents (Montreal); metals (Ag, Cd, Cu, Pb, Zn; As)	Houde et al. (2014); Bruneau et al. (2016); Giraudo et al. (2016)
Brown trout *Salmo trutta*	wild fish, caught along metal contamination gradient; RNA-seq; gill, gut, kidney, liver	metals (Cd, Cu, Ni, Pb, Zn)	Webster et al. (2013)
Three-spined stickleback *Gasterosteus aculeatus*	wild fish, caught from reference and Cu-contaminated stream exposed in laboratory to Cu (4 d); microarray; liver	metal (Cu)	Webster et al. (2017)
Freshwater pearl mussel *Margaritifera margaritifera*	wild mussel, collected up- and down-stream from known dump site; RNA-seq; kidney	metals (Cd, Cr, Ni, Zn)	Bertucci et al. (2017)

Table 4-6: Effects of metals at the population and community levels[16]

- Structural components of ecosystems (communities, the biotic components of an ecosystem; populations, the members of a species that occupy the same habitat and can possibly interbreed) are often considered the most sensitive indicator of disturbance.
- Chronic exposure to low levels of contaminants often results in severe effects on populations and communities in aquatic environments. Community and population data are essential for the monitoring of these effects.
- 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.
- 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 to be of limited usefulness for the detection of community changes.

  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 indicators of the state of a biological 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.[17] Such explanations remain speculative, however, since in most field studies the chemistry of the exposure medium (sediments, water column) was not sufficiently characterized.

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[16] The information in this table is taken from the Executive Summary of the MEND (1993) report.

[17] The discrepancy may also be related to the presence in the real world of niches or refuges where metal exposure is lower than that determined by bulk analyses of water or sediment.

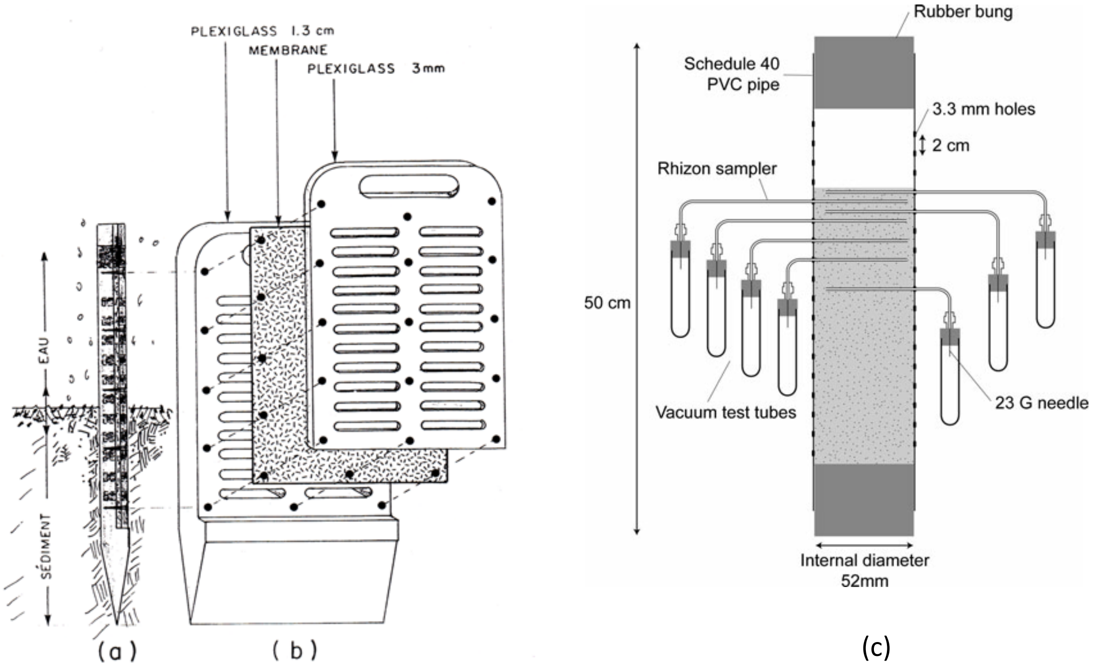

Figure 4-1: (a)(b) Typical dialysis-array sampler (pore-water peeper); (c) Typical rhizon deployment with sediment core (from Shotbolt (2010)).

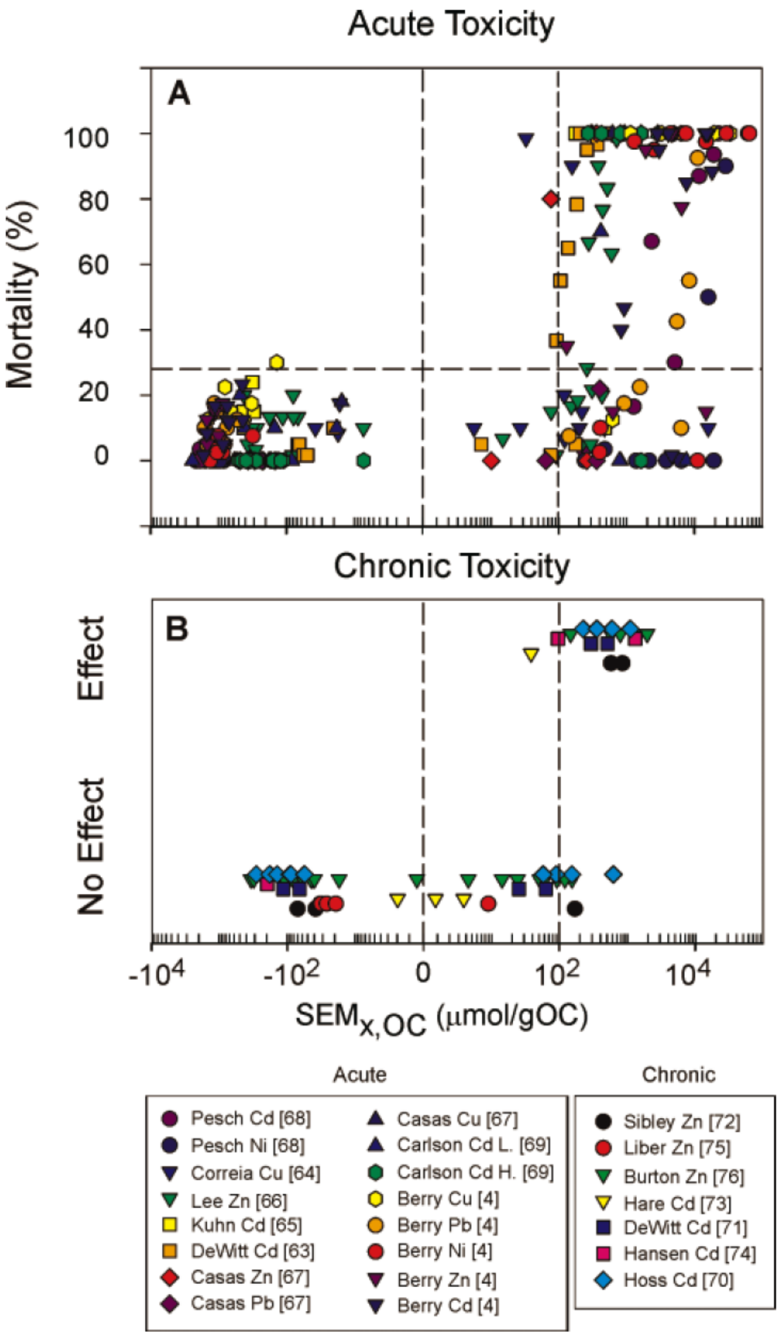

Figure 4-2: Toxicity data versus organic carbon–normalized excess simultaneously extracted metals concentration (SEM*$_{x,OC}$), plotted using a positive and negative log scale. (A) Mortality for acute exposures. (B) Chronic toxicity data identified, with either no effect or effect observed.

Figure reproduced from Di Toro et al. (2005), with permission from J. Wiley & Sons Publishers.

Note the existence of a threshold value of the SEM$_{x,OC}$ index, below which toxicity is not observed.

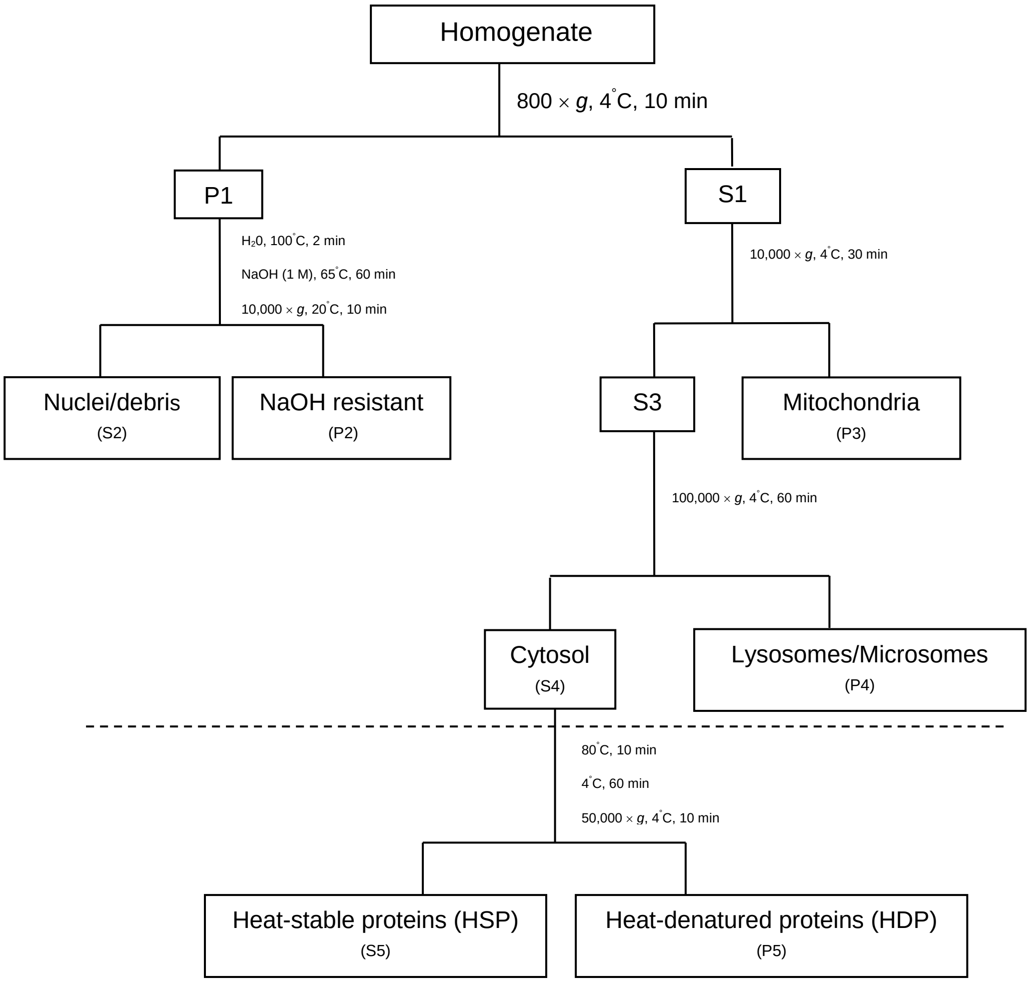

Figure 4-3: Schematic illustration of a subcellular fractionation procedure used to separate tissue homogenates into operationally-defined fractions. The fractions are numbered, with S = supernatant and P = pellet.

5 Relative Sensitivity of Different (Aquatic) Organisms to Metals

5.1 Introduction

In considering the potential effects of metals on the biota that colonize subaqueous disposal facilities, one might well ask what organisms are most sensitive to metals and are likely to be adversely affected, including aquatic organisms (e.g. aquatic plants, benthic invertebrates, fish) and non-aquatic organisms that feed on, reside in, or transit through the system (e.g. birds, mammals, etc.). Addressing this question is not straightforward, however, since some organisms may show marked sensitivity to one metal but tolerance to others. In other words, the answer to the question will vary from one ore body to another (or from one mine operation to another), as a function of the specific metals (and their form) present in the tailings. Note, too, that these differences in sensitivity to various metals are not necessarily the same for all target species. One way of addressing the question is to examine the 'species sensitivity distributions' or SSDs that are available for many of the metals of interest, both essential and non-essential.

5.2 Species sensitivity distributions (SSDs)

In developing water quality guidelines or water quality criteria, many jurisdictions have adopted an approach based on laboratory toxicity tests run under controlled conditions according to standard test procedures. In some countries including the USA, the tests are designed to determine short-term acute toxicity, whereas in other jurisdictions such as the European Union, the emphasis is on chronic toxicity. In Canada, both acute and chronic toxicity data may be used (CCME 2007). Canada has a list of preferred toxicity measures (CCME 2007), with the recommended approach being long-term exposures where "the EC_x value can be derived by regression analysis of the toxicological data and can be demonstrated to be at or near the no-effects threshold". Less preferred endpoints can be added to the dataset in the following order: $EC_{10}/IC_{10} > EC_{11-25}/IC_{11-25} >$ MATC > NOEC > LOEC > nonlethal $EC_{26-49}/IC_{26-49} >$ nonlethal EC_{50}/IC_{50}.[18]

The SSD can be visualized as a cumulative distribution function, where the effect concentrations derived from statistical analysis of validated acute or chronic toxicity tests are plotted in ascending order (Figure 5-1). The toxicity data for individual species are used to populate a species sensitivity distribution, which is then used to determine a threshold concentration designed to protect a given percentage of the species, usually 95% or 99%; this 'Hazardous Concentration' is expressed as the HC_x value (where 'x' is often 5, meaning that 5% of the species would be at risk) – see Figure 5-1. This approach assumes that HC_x values estimated from a subset of species will be acceptably protective of most species, both tested and untested, and that species protection confers ecosystem protection.

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[18] EC = 'effective concentration', IC = 'inhibitory concentration', and the subscript number (e.g., EC_{10}) refers to the percentage of test organisms that are affected. MATC = Maximum Allowable Toxicant Concentration, the geometric mean of the lowest exposure concentration that causes a statistically significant adverse effect (LOEC) and the highest exposure concentration where no statistically significant effect is observed (NOEC) in a chronic test.

For metals, much of the pioneering work using SSDs has been carried out in the European Union, as a means of estimating the Predicted No-Effect Concentrations or PNECs needed for their environmental risk assessments. In most cases, the metal concentrations present in the toxicity tests have been 'bioavailability corrected', to take into account the influence of factors such as pH, water hardness or metal complexation; this correction often involves the BLM approach that is described in section 2.2.

The use of the SSD approach for risk analysis is not without its critics (Forbes and Calow 2002; Duboudin et al. 2004; CCME 2005), but for the purposes of the present review, species sensitivity distributions do offer a visual means of identifying sensitive and more tolerant species. In a typical plot, the most sensitive species lie in lower left quadrant and the least sensitive species are found in the upper right quadrant (Figure 5-1). Examples of SSD plots for the chronic toxicity of individual metals can be found in Appendix C for Ag, Cd, Cu, Ni and Zn. For each metal, the most and least sensitive species have been compiled in Table 5-1. Small crustaceans (e.g., daphnids, amphipods) and unicellular algae appear frequently in the 'most sensitive' column, whereas the 'least sensitive' column includes a greater variety of organisms.

The sensitivity of crustaceans was also noted by Zheng et al. (2015), who collected and screened acute toxicity data from the U.S. Environmental Protection Agency's ECOTOX database and other sources for Cd, Cu, Cr(VI), Hg, Pb and Zn. The authors constructed individual SSD plots for each metal, for cladocerans and fish, as well as separate SSD plots for invertebrates and vertebrates; in general, invertebrates were more sensitive than vertebrates, although this distinction was not significant for Pb and Hg. The HC_5 values of the six metals decreased in the sequence Zn > Pb > Cr > Cd > Hg > Cu, indicating toxicities in opposite order.

Body size is one of the many factors that are thought to influence the sensitivity of organisms to metals (Newman and Heagler 1991; Brix et al. 2001), with small organisms (with a high ratio of surface area to volume) tending to be more sensitive. At first glance, the appearance of many small organisms in the 'most sensitive' column is consistent with this idea, but as explained below, this trend is likely at least partly an artifact because the range in size of test organisms is narrower than the range for natural aquatic biota. In addition, some of the toxicity tests are performed on early life stages (e.g., juvenile fish rather than adult fish), and in such cases the differences in size between a crustacean and a fish are less important than suggested by a comparison of mature life stages.

5.3 Limitations of the SSD approach

The SSD plots in Appendix C are all derived from standardized laboratory toxicity tests, with the test species having been chosen to meet the criteria of the relevant regulatory organization. These criteria vary somewhat from one jurisdiction to another. For example, in the European Union, 'reliable datasets' must comprise NOEC or EC_{10} values from at least 10 species belonging to at least 8 taxonomic groups, although preferably they should include more than 15 species (European Commission 2011). For EU freshwater systems, taxa deemed to be representative include fish; a second family in the phylum Chordata (e.g. fish, amphibian, etc.); a crustacean; an insect; a family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.); a family in any order of insect or any phylum not already represented; algae; higher plants. For Canadian fresh waters, the CCME requires three fish species (1 salmonid, 1 non-salmonid), three aquatic invertebrates, including ≥ 1 planktonic crustacean, and one vascular plant or alga.

Within the bounds of these criteria, aquatic toxicity tests are understandably biased towards organisms that can be cultivated easily and reproducibly in the laboratory, can be grown in standard growth media, and can be maintained in sufficient numbers to run multiple toxicity tests (and many of them are small). Note too that some authors have argued that the distribution of species used in a SSD should, in principle, reflect the natural distribution of species among primary producers, herbivores and carnivores (i.e., 64:26:10; Forbes and Calow (2002)). In contrast, for most SSDs used in the published literature to estimate HC_5 values, the average distribution is closer to 33:33:33, presumably reflecting the availability of standardized toxicity testing protocols. In other words, primary producers are under-represented and carnivores (fish) tend to be over-represented.

Finally, all the toxicity tests that underpin the SSDs in Appendix C were performed with dissolved metals. As discussed in Section 3.2, some metals and oxyanions can be taken up through the diet. If dietary uptake contributes to metal toxicity in the real world, some 'shuffling' of the SSD plots and a shift to lower HC_5 values might be expected. This possibility was examined for Cd by Mebane (2010), who compared the HC_5 value derived from laboratory testing to Cd concentrations that had been shown to have effects in ecosystem experiments and field studies. In a reassuring result, few adverse effects were observed in ecosystem experiments when the ambient dissolved Cd concentrations were less than the HC_5 value.

Van de Perre et al. (2016) performed a similar analysis for Zn, in which they calculated an HC_5 value for planktonic species on the basis of chronic toxicity tests carried out on individual planktonic species, and then determined if this value was protective for the planktonic community in mesocosms which were run at three Zn concentrations ([Zn] = background concentration, HC_5 value or HC_{50} value). The NOEC for the planktonic community in the mesososms was 75 µg Zn/L, a value similar to the HC_5 value calculated from the single species tests, suggesting that the HC_5 value was indeed protective of the community. In the mesocosms run at the HC_{50} value (300 µg Zn/L), there was a significant reduction in the number of cladocerans, which resulted in an increase in the abundance of rotifers, ciliates and phytoplankton, and the authors observed a shift in the phytoplankton community from grazing-resistant to edible species. The increase in phytoplankton abundance was not predicted by the SSD that had been used to calculate the HC_5 value; according to the SSD, phytoplankton should have been the most sensitive group. The authors concluded that "although the HC_5 estimated from the bioavailability-normalized SSD was overall protective for the plankton community, the SSD was not able to correctly predict the species sensitivity ranking within their community context at the HC_{50}" due to changes in grazing pressure.

This observation is a good example of how a metal, or indeed any environmental perturbation, can cause indirect effects that are not predictable when one considers only the sensitivity of individual species to the metal. Similar food-web mediated results have been reported for the whole-lake acidification experiments carried out in the Experimental Lakes Area (Schindler 1988) and for metal-contaminated lakes in the Rouyn-Noranda area in northern Quebec (Campbell et al. 2006; Rasmussen et al. 2008).

Table 5-1: Compilation of the least and most metal-sensitive species for Ag, Cd, Cu, Ni, Pb and Zn, based on an examination of the species sensitivity distributions (SSDs) for each metal.[19]

Metal	Most sensitive	Least sensitive	Reference
Ag (chronic)	algae some fish	insects macrophytes	Maycock et al. (2010)
Ag (chronic)	crustacean (*Ceriodaphnia dubia*) duckweed (*Lemna gibba*) some fish (rainbow trout)	crustacean (*Hyalella azteca*) insect larva (*Chironomus tentans*) some fish (smallmouth bass)	CCME (2015)
Ag (acute)	alga (*Chlamydomonas reinhardtii*) crustacean (*Ceriodaphnia dubia*) crustacean (*Daphnia magna*)	insect larva (*Tanytarsus dissimilis*) crayfish (*Orconectes immunis*) snail (*Aplexa hypnorum*)	CCME (2015)
Cd (chronic)	crustacean (*Daphnia magna*) crustacean (*Hyalella azteca*)	annelid worm (*Aelosoma sp.*) some fish (fathead minnow)	Mebane (2010)
Cu (chronic)	alga (*Chlorella sp.*) euglenid (*Trachelomonas sp.)* some fish (juvenile sturgeon)	mould (*Saprolegnia sp.*) macrophyte (*Lemna minor*) amphibian (*Ambystoma opacum*)	ANZECC and ARMCANZ (2016a)
Ni (chronic)	crustacean (*Ceriodaphnia dubia*) crustacean (*Ceriodaphnia quadangula*) mollusc (*Lymnaea stagnalis*)	toad (*Bufo terrestris*) rotifer (*Brachionus calyciflorus*) insect larva (*Chironomus tentans*)	NiPERA (2015)
Pb (chronic)	molluscs (*Lymnaea stagnalis*; *L. palustris*) some fish (common carp)	algae (*Pseudokirchneriella subcapitata*; *Scenedesmus sp.*) some fish (fathead minnow)	Grosell et al. (2006)
Pb (chronic)	molluscs (*Lymnaea stagnalis*; *L. palustris*) rotifer (*Philodina rapida*) crustacean (*Hyalella azteca*)	mayfly (*Baetis tricaudatus*) some fish (fathead minnow, rainbow trout)	DeForest et al. (2017)
Zn (chronic)	alga (*Pseudokirchneriella subcapitata*) crustacean (*Daphnia magna*) crustacean (*Hyalella azteca*)	mollusc (*Dreissenia polymorpha*) some fish (brook trout; mottled sculpin)	Personal communication (Eric Van Genderen, IZA)
Zn (chronic)	alga (*Pseudokirchneriella subcapitata*) alga (*Chlorella sp.*) crustacean (*Ceriodaphnia dubia*)	mollusc (*Physa gyrina*) mollusc (*Echyridella menziesii*) some fish (brook trout)	ANZECC and ARMCANZ (2016b)

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[19] The SSD plots are provided in Appendix C. In some cases the species names are indicated in the SSDs, but in others the data points are labelled at the phylum, class or family level.

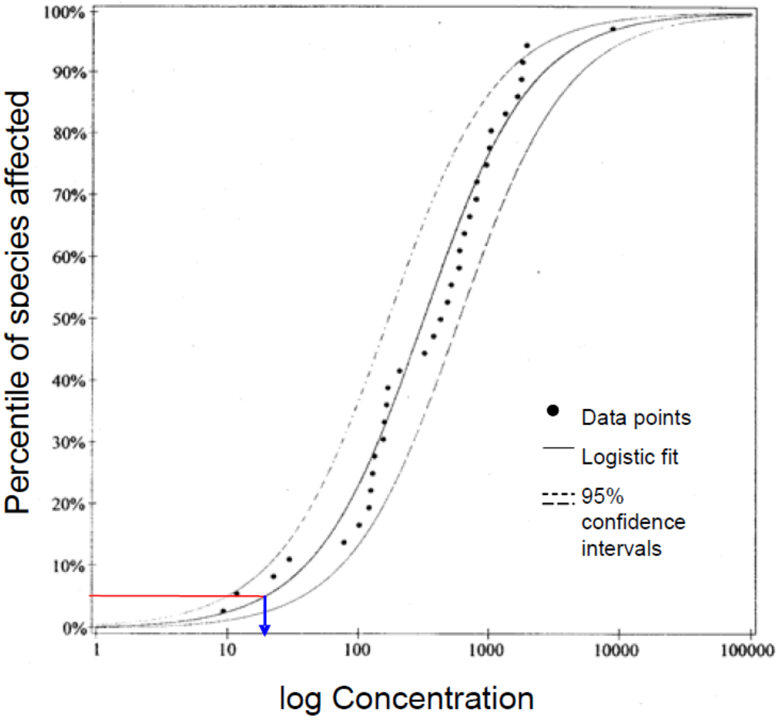

Figure 5-1: Generic species sensitivity distribution (SSD) plot.
(example taken from CCME (2007), showing the derivation of the HC_5 value)

6 Oxyanions

6.1 Overview

6.1.1 Environmental chemistry

In the context of this report, the elements considered are arsenic (As), molybdenum Mo), antimony (Sb) and selenium (Se)[20]. With reference to the chemical periodic table, As and Sb occur in group 15 (as does P), Mo occurs in group 6 (as do Cr and W), and Se occurs in group 16 (together with S and Te); elements that co-occur in the same periodic table group tend to behave at least somewhat similarly in the environment and in living organisms.

One of the properties of these elements that is common across groups is their existence in multiple oxidation states, e.g.:

- arsenic: As(-I), As(I), As(III), As(V)
- antimony: Sb (III), Sb(V)
- molybdenum: Mo(IV), Mo(V), Mo(VI)
- selenium: Se (−II), Se(0), Se(IV), Se(VI)

Unlike complexation reactions, which for most cations can be considered to be rapid (allowing equilibrium to be approached in natural waters), changes between oxidation states, i.e. 'redox reactions', are often slow. Such couples as As(III/V) and Sb(III/V) are frequently not at equilibrium and individual species can be found at concentrations that differ markedly from those predicted thermodynamically. However, many redox reactions can be microbiologically catalyzed, in which case the reaction rate will be determined by microbial growth and metabolic activity. For trace concentrations at pH 8, the sequence of redox reactions follows the approximate order $O_2 > NO_3^- >$ Mn(IV) > Se(VI) > Fe(III) >As(V) > $SO_4 \approx$ Mo(VI) (Smedley and Kinniburgh 2017).

Another commonality among these elements is the presence of methylated forms (for As, Sb and Se). These are not metal complexes, where the cation and the ligand are bound together by a coordinate bond, but rather true organometallic molecules where the non-metallic element and the methyl group are linked by covalent bonds (both as $M\text{-}O\text{-}CH_3$ bonds and direct $M\text{-}CH_3$ bonds). Because of the presence of a covalent bond, these methylated forms are much more stable than typical metal cation complexes. In addition to methylated forms, some of the oxyanions (e.g., As and Se) can also be incorporated into natural biomolecules such as arsenosugars and selenoproteins.

From a geochemical perspective, the oxyanions differ markedly from the metal cations that have been discussed to this point. All the oxyanions can exist as oxyanions, which are subject to rapid protonation and deprotonation reactions, depending on the pH (the dominant forms in neutral solution are shown below):

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[20] The term 'metalloid' is sometimes used to refer to As, Sb and Se, even though Se is not a true metalloid. All four elements share the property of existing as oxyanions.

- arsenic: arsenite ($As(OH)_3^0$); arsenate (AsO_4^{3-})
- antimony: antimonite ($Sb(OH)_3^0$); antimonate ($Sb(OH)_6^{1-}$)
- molybdenum: molybdate (MoO_4^{2-})
- selenium: selenite ($SeO_2(OH)^{1-}$; SeO_3^{2-}); selenate (SeO_4^{2-})

All four oxyanions are known to adsorb onto Fe oxyhydroxides (Groenenberg et al. 2012; Pérez et al. 2014) and onto other metal oxides (Al, Mn) (Smedley and Kinniburgh 2017). Most of these adsorption studies have been performed in the laboratory, but Feyte et al. (2010) collected authigenic Fe oxyhydroxides from lake sediments, using Teflon sheets (Belzile et al. 1989), and demonstrated that As, Mo and Sb were preferentially concentrated in the Fe(III) phase in the oxic sediments rather than the sedimentary organic matter. The degree of oxyanion adsorption tends to increase as the ambient pH decreases, which is the opposite trend to what is generally observed for cations. Accordingly, As, Mo, Sb and Se tend to be more mobile at circumneutral pH values than under acidic conditions (Lindsay et al. 2015), whereas the opposite behaviour is observed for cations. In addition to the pH effect, oxyanion mobility will also be affected by the redox potential. For example, under conditions favouring the reductive dissolution of iron oxyhydroxides, pore-water concentrations of arsenic (Martin and Pedersen 2002; Senn and Hemond 2002; Omoregie et al. 2013) molybdenum (Chappaz et al. 2008a) and selenium (Janz et al. 2014) have been shown to increase. Given the affinity of the oxyanions for redox-sensitive sorbents (Mn(IV); Fe(III)) and their own redox sensitivity, the presence of microbiologically labile organic matter in the upper strata of submerged tailings and the concomitant demand for electron acceptors will likely have a strong influence on their mobility.

As is the case for many of the metal cations, the oxyanions also react with reduced sulphur in sediments (either to precipitate as sulphides or to adsorb onto authigenic sulphides).

- arsenic: $As_2S_3(s)$; $FeAsS(s)$ (arsenopyrite)
- antimony: $Sb_2S_3(s)$
- molybdenum: $MoS_2(s)$

Reaction with reduced sulphur tends to immobilize As, Sb and Mo, although the actual mechanism of the reaction with Mo is uncertain (Smedley and Kinniburgh 2017).

Another common (and regrettable) theme for the oxyanions is the lack of reliable thermodynamic and kinetic data, notably for Sb and Mo, which obviously hinders the use of chemical equilibrium models to calculate their theoretical speciation in pore waters or in the overlying water column, and to predict their environmental fate and transport. In addition, quoting Smedley and Kinniburgh (2017) with respect to thermodynamic databases, “Many of the solid phases in the databases are of dubious significance when it comes to modelling low-temperature environments, particularly when considering relatively short timescales”.

6.1.2 Interaction with living organisms

Turning now to the interaction of oxyanions with living organisms, as a complement to the earlier discussion of cation-organism interactions (Sections 2 and 3), elements such as As, Mo, Sb and Se clearly do not conform to the Biotic Ligand Model (BLM), since they exist in natural waters as oxyanions rather than as free metal cations[21]. In addition, the mechanisms by which they are taken up by living organisms are less well understood than is the case for (essential) metal cations. Intuitively, as an analogy to the free-ion model for cations, one might have expected a free-anion approach to work for the oxyanions, but this idea has not been tested.

One trans-membrane transport mechanism that has been studied in some detail involves aquaporins. Aquaporins are protein-based channels, members of the Major Intrinsic Proteins super-family (MIPs), which allow the bidirectional passage of water molecules across biological membranes. Since their discovery in erythrocytes and renal tissue (Denker et al. 1988), aquaporins have been found in all animal taxa studied to date (Campbell et al. 2008), and are also present in plasma and intracellular membranes of higher plants (Maurel et al. 2009).

In addition to water, some types of aquaporins may facilitate the trans-membrane transport of non-polar solutes, such as urea or glycerol, the reactive oxygen species hydrogen peroxide, gases such as ammonia, carbon dioxide and nitric oxide, and neutral polyhydroxy species (e.g., $As(OH)_3$, $Sb(OH)_3$) (Bienert et al. 2008a; Bienert et al. 2008b; Yang et al. 2012; Zangi and Filella 2012; Mukhopadhyay et al. 2014). It seems that $As(OH)_3$ and $Sb(OH)_3$ behave as inorganic molecular mimics of glycerol, which allows them to enter and exit cells through aquaporin channels. Since the distribution of a given oxyanion among various polyhydroxy species will normally be pH sensitive, it follows that their bioavailability may also be influenced by the ambient pH. For example, the proportion of the neutral $M(OH)_3^0$ species is close to 100% in the pH range from 3 to 7 for arsenite but drops to 66% at pH 9. In contrast, for antimony in the reduced Sb(III) oxidation state, the $M(OH)_3^0$ form dominates over a very wide pH range, from 1 to 11 (Baes and Mesmer 1976). Both arsenate and antimonate are reduced when they enter living cells, which facilitates their subsequent elimination from the cells via membrane-bound aquaporins (i.e., a detoxification mechanism). In effect, aquaporins operate as bidirectional channels, which allow the passage of solutes by simple diffusion down a concentration gradient; in other words, they may function both as toxicity and detoxification pathways (Tamás 2016).

Some oxyanions may also cross biological membranes via anion transporters. For example, arsenate and antimonate (AsO_4^{3-}, SbO_4^{3-}) are known to enter biological cells via transporters that normally supply the cells with phosphate, whereas selenate (SeO_4^{2-}) can masquerade as sulphate and enter via a sulphate transporter. It follows that the rate of uptake of arsenate or antimonate will be affected by the ambient phosphate concentration, and that selenate uptake will be influenced by the ambient sulphate concentration. Zangi and Filella (2012) provide a useful review of the transport routes of oxyanions into and out of living cells and highlight the knowledge gaps that still prevail.

A corollary of our relative ignorance of the bioavailability of oxyanions in the sediment/tailings environment is that there is a corresponding lack of biomonitoring data. One recent exception is the work by the Ruder Bošković Institute in Croatia (Dragun et al. 2013; Dragun et al. 2018; Krasnići et al. 2018), where a team of researchers has been exploring the use of subcellular fractionation techniques to

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[21] Note that in the case of metal salts (both for cationic metals and 'metalloids'), it is assumed that the salt will fully dissociate when dissolved in water. In other words, uptake of the metal or oxyanion will be independent of its original 'partner' in the solid salt.

isolate the soluble metals and oxyanions in various fish and invertebrate species. They have used this 'cytosolic' concentration as an indication of the prior oxyanion exposure of the biomonitor species. They have collected biomonitoring species along suspected contamination gradients and reported values for As, Mo, Sb and Se (as well a whole suite of cationic metals) in fish tissues (gills; livers) and whole gammarids.

In the following sections, some relevant information about the biogeochemical behaviour of the oxyanions at the sediment-water or tailings-water interface has been summarized, in an element-by-element approach. Useful review papers for each oxyanion have been summarized in Table 6-1.

Table 6-1: Compilation of useful review papers for the oxyanions As, Sb, Mo and Se

Reference	**As**	**Sb**	**Mo**	**Se**
Groenenberg, J.E., Dijkstra, J.J., Bonten, L.T.C., de Vries, W., Comans, R.N.J., 2012. Evaluation of the performance and limitations of empirical partition-relations and process based multisurface models to predict trace element solubility in soils. Environ. Pollut. 166, 98-107.	X		X	X
Nordstrom, D.K., 2011. Hydrogeochemical processes governing the origin, transport and fate of major and trace elements from mine wastes and mineralized rock to surface waters. Appl. Geochem. 26, 1777-1791.	X	X	X	X
Lindsay, M.B.J., Moncur, M.C., Bain, J.G., Jambor, J.L., Ptacek, C.J., Blowes, D.W., 2015. Geochemical and mineralogical aspects of sulfide mine tailings. Appl. Geochem. 57, 157-177.	X	X	X	X
Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem. 17, 517-568.	X			
Filella, M., Williams, P.A., Belzile, N., 2009. Antimony in the environment: knowns and unknowns. Environ. Chem. 6, 95-105.		X		
Smedley, P.L., Kinniburgh, D.G., 2017. Molybdenum in natural waters: A review of occurrence, distributions and controls. Appl. Geochem. 84, 387-432.			X	
Sharma, V., McDonald, T., Sohn, M., Anquandah, G.K., Pettine, M., Zboril, R., 2015. Biogeochemistry of selenium. A review. Environmental Chemistry Letters 13, 49-58.				X
Zangi, R., Filella, M., 2012. Transport routes of metalloids into and out of the cell: A review of the current knowledge. Chem. Biol. Interact. 197, 47-57.	X	X		
Mukhopadhyay, R., Bhattacharjee, H., Rosen, B.P., 2014. Aquaglyceroporins: generalized metalloid channels. Biochim. Biophys. Acta 1840, 1583-1591.	X	X		
Yang, H.C., Fu, H.L., Lin, Y.F., Rosen, B.P., 2012. Pathways of arsenic uptake and efflux. Curr. Top. Membr. 69, 325-358.	X			
Tamás, M.J., 2016. Cellular and molecular mechanisms of antimony transport, toxicity and resistance. Environ. Chem. 13, 955-962.		X		
Environment & Climate Change Canada, Health Canada, 2017. Screening Assessment – Selenium and its Compounds, Government of Canada, Ottawa, ON, Canada, Report No. En14-303/2017E-PDF, 142 p.				X

6.2 Arsenic

Arsenic is often present in sulphide ores, notably those bearing gold. Once oxidized, its geochemical mobility increases but the oxidized forms have a marked affinity for iron oxyhydroxides, with arsenate binding more strongly and over a lower pH range than does arsenite. However, arsenic is released back into the pore water under conditions favouring the reductive dissolution of the Fe(III) phase (Omoregie et al. 2013). Under these conditions, and in the presence of dissolved sulphide, mono- and dithio-arsenates may also be formed, although these thioarsenicals proved unstable in the presence of sulphur-oxidizing bacteria and were oxidized to arsenate (Fisher et al. 2008). In this connection, Couture and Van Cappellen (2011) have recently reassessed the very complicated role of reduced and elemental sulphur in arsenic geochemistry.

Fawcett et al. (2015) carried out a particularly thorough study of the behaviour of arsenic (and antimony) in the sediments and sediment pore water at sites downstream from the Giant Mine in the Northwest Territories. Using advanced X-ray spectroscopic techniques (XANES and EXAFS)[22] to distinguish between As(III) and As(V) phases in the sediment cores, they observed that As(V)–O phases decreased in relative abundance with depth whereas an As(III)–S phase appeared at depth; arsenopyrite (As(-I)–S bond) and an As(III)–O phase were detected at all depths. Since As(III) bound to S was not present in the original ore, Fawcett et al. (2015) deduced that, where found at depth in the downstream sediments, the As(III)–S phase must represent a secondary phase. Such phases could result from As adsorbing to the surface of a sulphide, such as pyrite, or from the precipitation of an As(III) sulphide phase. In the sediment pore water, As(V) was the dominant form under oxic conditions but was replaced by As(III) in the anoxic zone.

In a complementary study, Arsic et al. (2018) combined diffusive gradients in thin films (DGT) and diffusive equilibration in thin films (DET) to provide a high resolution *in situ* comparison of arsenic, antimony and iron behaviour in a contaminated wetland sediment[23]. The sediment was studied in mesocosms, with an emphasis on the oxic-anoxic vertical transition. On passing from oxic to anoxic conditions, Fe(II), As(III) and As(V) were released from the sediment to the water column, consistent with As release being linked to the reductive dissolution of iron(III) oxyhydroxides (cf. Martin et al. (2003c)).

One of the sites studied by Fawcett et al. (2015) was populated by *Equisetum fluviatile* (a rooted aquatic known as the common horsetail). At this site both As and Sb were associated with detrital organic material and appeared to be mobile in the root zone. The authors concluded that oxygen diffusing from the roots to the sediments was promoting the oxidation of the secondary sulphide phases, causing acidification of the rhizosphere and leading to mobilization of As, S^{2-} (and Sb). However, in the zone below active plant growth, As (and Sb) were present primarily as inorganic phases, leading the authors to suggest that As might be released upon plant death and then reprecipitated.

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[22] XANES = X-ray Absorption Near Edge Structure; EXAFS = Extended X-Ray Absorption Fine Structure.

[23] The DGT and DET techniques have proved to be particularly useful for the study of oxyanions (see Table 4-1).

6.3 Antimony

Arsenic and antimony often co-occur at mining sites, and neither element has a known biological role. For antimony, Sb(III) and Sb(V) are the most common oxidation states, but distinguishing between the two is not straightforward (Filella et al. 2009; Daus and Hansen 2016). With O_2 as the sole oxidant, oxidation of Sb(III) appears to be extremely slow in homogenous aqueous solutions under environmental conditions. In a laboratory study, no significant oxidation of Sb(III) with O_2 was observed within 200 days in the pH range of 3.6–9.8 (Leuz and Johnson 2005), but in a subsequent paper Leuz et al. (2006) reported that the oxidation rate was greatly accelerated in the presence of Fe(II) in what they referred to as an 'iron-mediated' oxidation. Acceleration of the oxidation of Sb(III) → Sb(V) has also been reported in the presence of Mn oxides and humic acids (Filella et al. 2009).

Although As and Sb are in the same group in the periodic table and have similar electronic structures (ns^2p^3), they exhibit different geochemical behaviours (in their pentavalent forms). The dominant dissolved forms of Sb(III) and As(III) (i.e., $Sb(OH)_3$ and $As(OH)_3$ respectively) are recognized to be similar to each other at the structural and reactivity levels, and given their dissociation constants (pK_a) of 11.8 and 9.2 respectively, they both exist as neutral solutes in aqueous solutions with similar diameters (Daus and Hansen 2016; Tamás 2016). In contrast, the oxidized forms As(V) and Sb(V) are dissimilar; the former adopts a tetrahedral structure whereas the latter has an octahedral structure, and thus different geochemical and biological behaviours are expected. For example, Sb(III) binds more strongly to Fe(III) oxyhydroxides than does Sb(V), and over a wider pH range, whereas As(V) binds more strongly and over a lower pH range on the surfaces of iron oxide minerals than does As(III). Similarly, As and Sb have been shown to behave differently under anoxic conditions – in a study of the behaviour of the two elements in a contaminated wetland, As was released to pore water when Fe(III) oxyhydroxides were reduced, whereas Sb reacted with and was bound to reduced sulphur (Arsic et al. 2018). In this latter study, both Sb(III) and Sb(V) diffused out of the wetland sediment under oxic conditions, but under anoxic conditions the direction of the fluxes was reversed. Using solid-phase X-ray absorption spectroscopy (XAS), Bennett et al. (2017) detected an Sb(III)-S phase in the wetland sediment that increased in proportion with depth and with the transition from oxic to anoxic conditions. The DGT and DET profiles across the oxic-anoxic transition zone suggested an apparent decoupling of Fe(II) and Sb release. The authors concluded that under their experimental conditions, Sb mobilization was controlled by reactions with sulphur and/or organic carbon (e.g., formation of authigenic Sb_2S_3 or Sb(III) complexation by thiol functional groups).

Differences in geochemical behaviour for Sb and As were also noted in the study conducted by Fawcett et al. (2015) downstream from the Giant Mine (cited in section 7.2 for arsenic), where they explored the forms of Sb in the pore water and solid phase of their sediment cores. They noted that Sb(V)–O phases persisted down the core, unlike the case for As(V)–O phases, and a Sb(III)–S phase appeared deeper in the core in the anoxic zone (cf. the Bennett et al. (2017) results cited above). Similarly, in the pore water Sb(V) species were present in the oxic zone and persisted in the anoxic strata, whereas only As(III) was present in the anoxic pore waters. Fawcett et al. (2015) suggested that any Sb(III) had been removed from the anoxic pore water by precipitation as sulphides or adsorption to sulphides, and they noted that "oxidized species of Sb and reduced species of As can coexist in (pore water) solution".

6.4 Molybdenum

Molybdenum is a strongly chalcophile element, its main ore being molybdenite (MoS_2), but it is also recovered as a by-product of mining Cu porphyry deposits. Unlike arsenic and antimony, molybdenum is a biologically essential metal; it is a co-factor required for the functioning of many enzymes (>60), including notably those involved in nitrogen fixation and nitrate reduction (Smedley and Kinniburgh 2017). Molybdenum exhibits coordination numbers from 4 to 8 and exists in formal oxidation states from -II to VI, but Mo(IV), Mo(V) and especially Mo(VI) are the most important in the environment. In oxic waters, molybdate (MoO_4^{2-}) is the dominant species, with protonated forms only occurring at pH < 5, in relatively small proportions. Polynuclear species can form at low pH and at very high Mo concentrations, although Smedley and Kinniburgh (2017) suggest that "only in exceptional circumstances, as with acidic drainage from near Mo-rich mines and ore bodies, are the more exotic polynuclear species likely to be found". Under reducing conditions in the presence of sulphide, aqueous complexes with S are formed, with Mo assumed to have remained in the Mo(VI) oxidation state. These complexes include a sequence of thiomolybdates in which S^{2-} replaces the O^{2-} in $Mo(VI)O_4^{2-}$ in a stepwise manner ((MoO_3S^{2-}, $MoO_2S_2^{2-}$, $MoOS_3^{2-}$, MoS_4^{2-}) (Stockdale et al. 2008); the first three substitutions are relatively rapid but the last is very slow and equilibrium may not be achieved. Unlike molybdate, which is rather unreactive towards surfaces, thiomolybdates are relatively reactive (adsorption; reaction with natural organic matter, NOM) (Smedley and Kinniburgh 2017).

In terms of environmental emissions, long-range atmospheric transport from smelting and coal combustion can contribute to lake sediment Mo contents. Chappaz et al. (2008a) reported that Mo concentrations in lacustrine sediments from Quebec lakes located downwind from the Horne smelter in Rouyn-Noranda were 3 to 16 times higher than in the deeper, pre-industrial sediments. In this study of three lakes with seasonally anoxic bottom waters and one perennially oxic lake, the authors found no evidence for redistribution of Mo in the sediments during periods of anoxia. However, Mo profiles obtained from pore water peepers installed on three sampling dates in the perennially oxic basin revealed Mo redistribution after sediment deposition, as indicated by sharp Mo peaks below the sediment-water interface. The release of Mo at a depth of 1-2 cm was linked to the reductive dissolution of Fe(III) oxyhydroxides; the released Mo was then scavenged near the sediment-water interface by re-adsorption onto newly formed authigenic Fe oxyhydroxides, and also deeper in the sediments where pore-water sulphide concentrations were higher.

The inorganic geochemistry of Mo appears to be relatively well-defined, with iron oxyhydroxides playing a key role as adsorbent under oxic conditions and aluminum oxides acting in this role when the hydrous ferric oxides have disappeared.[24] Under reducing conditions, amorphous FeS constitutes the main reservoir of Fe and S, and Eh-pH calculations suggest that a coexisting mixed ferrous molybdenum sulphide phase ($FeMo_{0.6}S_{2.8}$) controls the dissolved Mo concentration (Smedley and Kinniburgh 2017).

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[24] These sorption reactions have been studied with the use of XANES and EXAFS, techniques that provide information about the oxidation state of the Mo atom and the nature of the donor atoms (bond lengths and nearest neighbour distances) and distinguish between inner- and outer-sphere binding) – Smedley and Kinniburgh (2017).

On the other hand, the role of binding to dispersed and reduced natural organic matter is much less clear. There is circumstantial evidence that Mo concentrations tend to increase in organic-rich and/or sulphide-rich fine-grained sediments, but the mechanism of binding between Mo and organic matter remains obscure. In their comprehensive review of the distribution of molybdenum in natural waters, Smedley and Kinniburgh (2017) conclude that "overall, it appears that Mo can bind to natural organic matter but it does not appear to be particularly strong or selective except perhaps under sulphide-rich conditions."

6.5 Selenium

An essential micronutrient, selenium participates in a variety of biological processes, including notably the protection of cells from oxidative stress; there are also reports that selenium can participate in the protection of cells from Cd and Hg toxicity (Scheuhammer et al. 2012; Ponton et al. 2016). At concentrations exceeding metabolic requirements, selenium can also induce toxicity in aquatic organisms, but it differs from the other oxyanions in that the range between sufficiency and toxicity is very narrow (Janz et al. 2014).

Selenium and sulphur tend to co-occur in the geosphere, notably in sulphur and coal deposits. Unlike the case for many metals and oxyanions, for which anthropogenic sources are dominant, total emissions of Se to the environment are dominated by natural sources (50-65%; Sharma et al. (2015)) such as crustal erosion, entrained sea spray, volcanoes (H_2Se) and notably the microbial generation of volatile organo-selenium species (dimethylselenide $(CH_3)_2Se$; dimethyldiselenide $(CH_3)_2Se_2$). Inorganic species present in freshwater environments include selenide Se(–II), elemental selenium Se(0), selenite Se(IV) and selenate Se(VI). Total dissolved selenium concentrations in uncontaminated fresh water are normally < 0.1 $\mu g \cdot L^{-1}$; under mild oxidizing conditions selenite should be the dominant species ($SeO_2(OH)^{1-}$; SeO_3^{2-}), whereas under strong reducing conditions and at pH values > 4 selenium speciation is dominated by selenide (HSe^{1-}). Detailed speciation *versus* pH plots can be found in Sharma et al. (2015).

As discussed for the other oxyanions, Se tends to adsorb onto Fe(III) and Al(III) oxyhydroxides under oxic conditions. Selenite is more strongly sorbed than selenate, and its sorption is strongly affected by the ambient pH (maximal in the pH range 3.0 to 4.0 and declining markedly as the pH increases above 7). In their comprehensive study of a northern coldwater ecosystem, Janz et al. (2014) reported that under suboxic conditions (1-2 cm below the sediment-water interface) increases in pore-water Se correlated with increases in pore-water Fe, suggesting release of Se by reductive dissolution of Fe oxyhydroxides. Under reducing conditions, selenite has been shown to be reduced to elemental Se by sulphide, cysteine and ascorbic acid; rates in laboratory studies were slow at circumneutral pH but more rapid under acidic conditions (Sharma et al. 2015). The reduction of oxidized forms of Se to insoluble Se(0) is likely a major factor in Se immobilization in lake sediments. In the Janz et al. (2014) study, approximately 50% of the sediment Se was in the form of elemental Se, although this ranged from 0% to 81% among samples. Other Se species detected in sediments, as determined by synchrotron-based XAS, included seleno-sulphides, selenite, and inorganic metal selenides.

Natural organic matter (NOM) also appears to be involved in selenium geochemistry. For example, Se concentrations in soil (Sharma et al. 2015) and sediments (Janz et al. 2014) appear to correlate directly with that of NOM concentrations, but the nature of the interaction between selenium and the NOM is unclear, as is the redox state of the selenium itself. It has been suggested that this association reflects not sorption *per se* but rather assimilation by primary producers and microorganisms (components of surface biofilm), and subsequent incorporation into the organic carbon component (living or dead) of the sediment (Janz et al. 2014). In this latter study, there was no relationship between Se concentrations in whole-sediment and Se concentrations in pore water, but selenite concentrations in whole-sediment were positively correlated with the pore-water Se content. The authors suggested that this correlation reflected an equilibrium partitioning relationship between adsorbed and dissolved selenite in the sediment. There was also a strong relationship between pore-water Se and Se in chironomids, the dominant detritivore in the studied ecosystem.

No discussion of selenium biogeochemistry would be complete without a consideration of its trophic transfer. The status of Se as the most environmentally problematic of the four oxyanions is linked inextricably both to the very narrow range between sufficiency and toxicity, and to its propensity for movement between trophic levels (see Section 3.2 for an earlier discussion of the dietary uptake of cations). Selenate and selenite are readily taken up by phytoplankton and periphyton, and transformed biochemically into organo-selenium molecules. When these primary producers are grazed by consumers, these molecules can then be transferred to higher tropic levels. Within living cells, Se is associated with amino acids, proteins, and metabolic intermediates; it is often incorporated into proteins as selenomethionine (SeMet), a substitution that tends to compromise the functionality of the protein. Using synchrotron-based XAS, an analytical technique that probes the local chemical environment of Se but does not identify a specific seleno-molecule, Janz et al. (2014) demonstrated that the absorption spectrum of the dominant form of selenium in the periphyton associated with the sediment surface corresponded to a SeMet-like species. A similar dominance of forms resembling SeMet was observed in chironomids, the dominant benthic detritivore, as well as in foraging and predatory fish species; since plants and bacteria, but not animals, are able to synthesize SeMet, these results clearly demonstrate the key role of the periphyton in initiating the dietary transfer of Se to higher tropic levels. This dietary transfer of organoselenium is particularly problematic for early life stages (embryos and larvae) of vertebrate animals (e.g., fish, birds, amphibians, reptiles) that reproduce by means of eggs that are hatched after they have been laid by the parent. In the aquatic environment, chronic exposure to selenium has been implicated in cases of fish extirpation and teratogenicity in fish-eating birds (Janz et al. 2010; Environment and Climate Change Canada and Health Canada 2017).

7 Information About Subaqueous Disposal of Mine Tailings

7.1 Regulatory guidance

Environmental regulations as they apply to mine sites are designed to achieve discharge limits, minimize human and ecological risks, restore acceptable site uses and ensure that mitigation costs are identified and there is no outstanding financial liability. Aspects that are subject to regulatory conditions and actions include health and safety, geotechnical stability, ML/ARD mitigation, discharge to the environment and reclamation requirements. Continual monitoring and maintenance is required to sustain the performance of engineered features, such as dams and spillways. Verification through monitoring is needed where there is uncertainty about future performance of mitigation and reclamation measures. Where containment and mitigation facilities need to be maintained or monitored, there is no walk-away solution and the short-term economic benefits of mining must sustain these long-term activities.

Regulatory guidance specifically regarding post-closure environmental performance of subaqueous disposal facilities is provided in the 'Guidelines for Metal Leaching and Acid Rock Drainage at Minesites in British Columbia' (Price and Errington (1998), which has been formally been adopted in British Columbia and Ontario and to varying degrees elsewhere in Canada.

> "All stages of research and planning should include consideration of the long-term performance of the underwater storage facility and the limitations, opportunities and cost effectiveness provided by different forms of pond management and post-closure mitigation. Modifications in operational pond management may be an effective means of avoiding uncertainty, additional research and security costs."
>
> "As nature reasserts itself, there are a number of possible mechanisms that may cause contaminant release. The current expectation is that the impact of processes like sedimentation and biological uptake on contaminant migration will be relatively small and that any potential problems can be prevented through additional mitigation. During the life of the mine, the operator must conduct test work to assess the potential for post-closure contaminant release and determine if additional mitigation is required."

Post-closure reclamation goals for water covers will depend on site attributes such as water depth, physical and chemical properties, associated drainage features and climate. Where there are no chemical restrictions, a flooded mine component will become an integral component of the post-closure landscape, contributing habitat, food and water storage. Shallow water bodies may be best suited for waterfowl, shorebird and wildlife habitat. Deeper ponds may support productive fisheries.

7.2 Differences between subaqueous tailings facilities and natural water bodies

The preceding parts of this report have dealt largely with the peer-reviewed scientific literature, with an emphasis on papers published since 2008-9 (date of publication of MEND Report 2.11.2(b)). In addition to this material, data from subaqueous disposal facilities across Canada in company and government reports and conference proceedings were provided, with the assistance of NRCan-CanmetMINING. A list of the reports, conference proceedings and BC-MEND ML/ARD slide presentations provided by NRCan-CanmetMINING can be found in Appendix D.

Before reviewing the information on subaqueous disposal (SAD) facilities and comparing this information with what is available on natural lentic systems, it is important to recognize that the focus of this report is on SAD facilities created with one or more water retaining dams. Some properties of SAD facilities, such as age, sediment mineralogy and physical composition, initial pore and surface water chemistry and presence of man-made structures, such as dams and runoff diversions, are completely different from those of natural water bodies. Other SAD properties have natural analogues, such as a lack of oxygen inputs during the winter and the onset of anaerobic conditions, but these conditions are more likely to occur in subaqueous tailings facilities.

There are a wide variety of types and forms of natural lentic water bodies, and their ecological and hydrological ‘performance’ will vary accordingly. Differences between SAD facilities and natural water bodies and where the SAD facility fits within the spectrum of natural water body conditions should be kept in mind when general reclamation goals and more detailed performance objectives and methods to increase performance are selected.

Some of the more obvious differences between SAD facilities and natural water bodies are summarized below.

Age:

- Closed SAD facilities and the surrounding closed mined landscape are often only years or decades old. Consequently, many properties are likely to be in state of flux and SAD facilities will be experiencing both local and larger scale changes to physical properties (e.g., settling), geochemistry (e.g., sulphide oxidation), hydrology (e.g., groundwater rebound), land use (e.g., mine reclamation), terrestrial vegetation (e.g., reforestation), soil development and aquatic ecology.
- Biological communities in natural water bodies, which may have developed since the last glaciation, would be expected to be much more mature than those in SADs, which are still in their infancy in terms of sediment and pore water diagenesis and inputs from adjacent land and water bodies.

Sediment mineralogy and physical composition:

- Tailings differ geochemically from normal sediment because mines target geochemically anomalous geologic materials with elevated concentrations of sulphide minerals or their oxidation products and of several trace elements. The physical and chemical properties of the submerged tailings also reflect the effects of crushing and grinding, the addition of reagents, as well as mineral alteration, removal and segregation during processing.
- Tailings in a single SAD facility may consist of a number of physically or geochemically different materials as a result of variability in the mine geology, separate disposal of different tailings,

segregation of the tailings after deposition, or because the processed ore came from more than one mine site. Sediments in a natural water body tend to vary in granulometry, with fine-grained particles being more prevalent in the deeper portions of the natural water body than in the areas closer to the shore. Something similar often occurs during slurry tailings disposal, with alluvial segregation resulting in the deposition of tailings sand near the deposition points and silt-sized tailings (slimes) in the centre or furthest areas of the impoundment. In some case, tailings are predominantly silt-sized either as a result of a fine grind or because the tailings were cycloned and the sand fraction was used for dam construction or backfill underground.

- Deposition of slurry tailings is typically from spigots located around the perimeter of the facility. Tailings deposition is moved around the perimeter to prevent tailings from building up in any one location. Changes to spigot locations create more heterogeneity, with subsequent spigoting events eroding past depositional layers or creating new layers with particle sizes and mineral deposition potentially different than those in the underlying layer.
- Sometimes, to maximize use of the storage space in the impoundment, tailings may also be deposited from a barge in the centre.
- Differences in geochemistry within a SAD facility may result from settling of higher density minerals (e.g., sulphide minerals) near the deposition points and lower density minerals (e.g., carbonate minerals) in the furthest areas of the impoundment. Differences in geochemistry may also result when different processing streams – low sulphide rougher and higher sulphide cleaner and scavenger streams, or desulphurized tailings and residual sulphides from desulphurized tailings – are deposited separately.
- Processing that includes roasting and pressure oxidation (e.g., Pueblo Viejo and Campbell mines) will produce a tailings geochemistry with trace elements occurring in oxidized iron(III)-based minerals, such iron oxides (Fe_2O_3), jarosite ($KFe_3(OH)_6(SO_4)_2$) and scorodite ($FeAsO_4{\cdot}2H_2O$). Subsequent reductive dissolution of the iron (III)-based minerals may release trace elements into the pore water.
- The sediment composition in a SAD facility may also include non-tailings materials, such as the residues from the treatment of wastewater streams (e.g., ARD treatment products) and metal-sulphate sludges produced by the neutralization of mill streams bearing sulphuric acid (Martin et al. 2003c) or solid phases that have precipitated from process water or other drainage inputs to the impoundment. Metal hydroxides precipitated after the mixing of different drainage chemistries may be sensitive to post-closure changes in pH (see section 7.3).
- The bottom sediments of natural water bodies reflect both allochthonous inputs (e.g., suspended sediments from the catchment) and autochthonous inputs (e.g., plankton settling out from the overlying water column). In young SAD facilities, the bottom 'sediments' will obviously be dominated by the tailings themselves and will tend, at least initially, to be poorer in natural organic matter and thus less able to support a heterotrophic microbial community. This impediment may be overcome with suitable amendments. For example, a heterotrophic microbial community was established in Highland Valley Copper tailings management facilities after 2-3 years when nutrients and a natural sediment inoculum were added, and after 4-5 years without inoculation (Heather Larratt, Personal communication, 2018-05).
- Oxidation of sulphide minerals in tailings is likely to result in the precipitation of secondary metal phases at or adjacent to the surface. The precipitated secondary metal phases are likely to include iron oxyhydroxides since iron is a major component of the most common sulphide minerals pyrite and pyrrhotite. Due to the near-neutral or basic pore-water pH, subaqueous tailings with high sulphide content have the potential to develop high concentrations of iron

oxyhydroxides that are conducive to the formation of iron cemented layers at or near to the tailings-water interface.

Pore and surface water chemistry:

- The physical and chemical actions of processing will create a pore and surface water chemistry that initially differs geochemically from that of natural lakes. The composition of process water will include processing chemicals, residues from blasting, and solutes released by the abrasion and processing of ore minerals.
- Processing chemicals include flotation agents (e.g., xanthates) and chelation agents (e.g., cyanide). Metal-bearing process reagents, such as copper sulphate, may increase metal concentrations. Ore separation processes are normally conducted at a basic pH and alkaline processing chemicals, such as lime, may be added for pH balance. Process reagents are usually added in small amounts.
- Solutes released by oxidation and dissolution during the abrasion and processing of ore minerals include sulphate from ores containing gypsum and anhydrite, and trace elements elevated in the ore (e.g., Mo at porphyry mines like Highland Valley Copper, Zn at Samatosum Mine and As at Equity Mine). Intermediate sulphur oxidation products called thiosalts (e.g., $S_2O_3^{2-}$, and $S_xO_6^{2-}$; $3<x<10$) are produced by oxidation in the processing of high sulphide ore (e.g., Kidd Creek, Strathcona and Brunswick Mines). Nitrogen species are derived from ammonium nitrate used in blasting and the degradation of cyanide species.
- Process water typically has a near-neutral or basic pH, due to the basic abrasion pH of ore minerals and the addition of alkaline processing chemicals. Exceptions to the basic nature of process water include process water rich in thiosalts. Typically, thiosalt-bearing process water will still be basic when it is discharged into a tailings facility, but subsequent oxidation of the thiosalts may decrease the water pH within the facility.
- During deposition of tailings within the impoundment, continual wetting with alkaline process water and the raised water table minimize tailings oxidation. Consequently, at some mines the chemistry of the water cover is acceptable for discharge while the mine is operating (e.g., Huckleberry). However, continual water reuse and recirculation may result in elevated concentrations while the mine is operating.
- Soon after processing and solute additions stop, attenuation, decomposition and dilution of solutes in the water cover often reduce concentrations sufficiently for the tailings water cover to meet the water quality criteria required for discharge.
- Solute concentrations in sediment pore water will also decrease after mine closure, but changes, especially at depth, are likely to be slower and significant improvements may take considerably longer to occur than in the water cover.
- Treatment may be required when it is necessary to discharge water and the water quality does not meet receiving environment discharge limits (e.g., fertilization of the water cover to increase biogenic scavenging (Highland Valley Copper); SO_2-air treatment to degrade cyanide (Equity and Red Lake); and ferric salt addition to co-precipitate arsenic (Eskay Creek and Red Lake mines)). Water treatment may occur during processing prior to tailings discharge, or within or below the SAD facility.
- The elevated concentrations of sulphide minerals and carbon process reagents, such as cyanide and xanthates, may increase the rate at which O_2 is consumed, reduce the depth of penetration of O_2 into sediment and increase the potential for reducing conditions at depth in the pore water of tailings compared to natural lake sediments. The presence of elevated nitrate from

blasting residue and cyanide degradation may increase the depth of oxidation (see Sections 4.4.1, 4.4.4).

Man-made features, such as dams, spillways and runoff diversions:

- Subaqueous disposal can be achieved using constructed dams, pits and natural water bodies such as lakes. SAD facilities created with one or more water-retaining features differ from natural lentic water bodies in having dams, spillways and in some cases runoff diversions, although natural water bodies may have these features as well due to the need for water management.
- SAD facilities that constrain the tailings and water cover with a mix of dams and higher ground exist in a wide range of topographical and hydrological situations. While there are examples of cross valley dams (e.g., Kemess and Samatosum mines), SAD facilities are generally placed in areas that minimize impacts on fish-bearing waters and the need for major stream diversion during tailings discharge. SAD dams and the material contained within are raised compared to the original ground and this will tend to reduce drainage inputs. The lack of stream flow into SAD facilities reduces sediment inputs that can contribute to development of a benthic community, limits the opportunity for arrival of colonizing species from off-site, and restricts oxygen inputs when there is ice coverage.
- Seepage loss will occur through dams whereas groundwater and runoff will enter SAD facilities from higher ground. Dams raise the level of the impoundment compared to the pre-mine topography, which may limit inputs of groundwater and surface runoff. Gravity-fed seepage collection ponds are usually constructed at the toe of dams so that seepage can be monitored and if necessary pumped back into the impoundment.
- The common engineering practice of constructing diversion structures may limit hydrological inputs from natural ground upslope from the catchment and the size of the contributing watershed. Diversion structures may be decommissioned after spillway construction if the quality of the water cover is acceptable for discharge.
- The limited hydrological inputs from the catchment (especially for surface water) will also likely affect the biological inputs and influence the rate and nature of colonization of the SAD.
- Inputs from catchments (e.g., nutrients, natural dissolved organic matter, suspended solids, terrestrial foliage) are known to vary as a function of land use within the catchment (Wetzel 2001b). Inputs from woody vegetation may be reduced by safety constraints on tree growth on dams, as well as the time required for a forest to develop on reclaimed mine land in the watershed upgradient from the SAD facility. However, grasslands take far less time to develop and inputs from herbaceous vegetation would be expected to compensate.
- Although the dams in SAD facilities usually include a relatively impermeable layer, such as a compacted till core, to minimize seepage losses and maintain flooded conditions, groundwater seepage through or in permeable strata under the dams may be a major portion of the discharge (e.g., Kemess Mine).
- Discharge of excess surface water is usually through a spillway built in bedrock for geotechnical reasons, around the edge of the dams. The spillway may have a gradient that impedes fish passage (e.g., Snip and Samatosum mines).
- Depth of the water cover will depend on the configuration of the facility and the discharge of the tailings. The typical objective is to achieve a water cover depth of 1 to 2 m throughout the facility, but the water cover depth is often considerably deeper in areas a long distance from the former points of tailings discharge. For example, at the Equity Mine the depth of the water cover

is 1.6 where the tailings were discharged and 7 m in the middle of the impoundment. Post-closure, some facilities may use water pressure or dredging to lower sub-aerial tailings below the height of the water cover (e.g., Falconbridge) or lower higher areas of the submerged tailings so the mine can to lower the height of the water cover and lessen the water volume (e.g., proposed at Equity Mine).

- The depth of the water cover will influence the ability of wind to induce resuspension of tailings and will also affect the degree of light penetration down to the biofilm growing on the tailings surface. The water cover should be deep enough to ensure that the tailings remain flooded during a long drought and to minimize entrainment and movement of tailings onto beaches by wave action and ice. Wave action depends on the fetch and wind speed and is difficult to predict (Yanful 2001).
- Tailings tend to compact after deposition. Post-closure settling and compaction may lower the height of the tailings relative to the height of the spillway and increase the depth of the water cover (e.g., Snip and Kam Kotia mines).
- Water levels will fluctuate in both the natural and man-made water bodies, in response to seasonal or inter-annual changes in water inputs, with obvious effects on the depth of the water column covering the bottom 'sediments'. In SAD facilities, control of the elevation of the water cover by periodic pumping rather than the height of the spillway may result in large fluctuations in water depth and the shoreline position. Depending on the bathymetry of the individual water body, these fluctuations may lead to the periodic exposure of the tailings to the atmosphere in the shallowest areas if the SAD facility is not properly designed.
- Low drainage input rates will increase water residence times in SAD facilities, whereas shallower depth and small volumes will decrease water residence times. With increased water residence times, there will be less dilution of process chemicals and less oxygen entering the facility during ice coverage.

Colonization and Diagenesis:

- Since SADs often have very small catchments in relation to their surface area, nutrient inputs can be low, sustaining oligotrophic conditions. Adsorption of phosphate on Fe oxyhydroxides at the tailings surface would also favour maintenance of an oligotrophic state.
- Deeper facilities may thermally stratify (Larratt and Ohata, 2013). Periodic mixing of stratified layers will resupply surface microflora with nutrients from deep water, maintaining productivity during the summer.
- Sedimentation of suspended solids may create a protective layer on top of the flooded tailings, potentially decreasing diffusion of oxygen into the wastes. Such a layer would also tend to reduce the diffusion of dissolved contaminants from the tailings into the overlying water cover and to lessen the contact of colonizing biota with the tailings. However, the limited catchment area will limit the inputs of suspended solids and hence the accumulation of natural sediments. Bioturbation and the associated mixing of the layer of natural sediments with the underlying tailings would obviously impede development of the protective layer.
- Depletion of dissolved oxygen (DO) is a common phenomenon in shallow ice-covered lakes and should be considered when setting ecological reclamation goals for tailings impoundments. The DO requirements of an aquatic organism will depend on the species, its life stage, the water temperature, and many other factors. Nevertheless, it is generally accepted that when DO levels drop below about 5 mg O_2/L, aquatic life is subject to stress. Fluctuations in DO can result from changes in inputs due to surface mixing and photosynthesis and losses due to bacterial

decomposition of organic matter and other oxidation reactions. Seasonal ice covers impose a barrier to O_2 entry, and depending on the duration and rates of oxygen consumption, may result in suboxic conditions within the water cover.

- Some tailings facilities may be designed to prevent colonization by fish, in part for regulatory reasons.
- Given the relative youth of mining water bodies, they will have initially limited additions of labile carbon to sediment from phytoplankton, zooplankton and aquatic plant growth. Oxygen in the sediment will be consumed by the oxidation of sulphide minerals, but oxidation of natural organic compounds and any residual process chemicals, such as xanthates and cyanide, is likely to be the dominant source of oxygen demand.
- Even if the metals present in submerged tailings prove to be biologically unavailable, the slow colonization of SADs and the presence of less than optimal benthic conditions (with respect to the availability of nutrients and natural organic matter, and also because of the grain size, grain sharpness and cohesion of the tailings) might well lead to an ‘impoverished’ benthic invertebrate community, i.e., one with fewer and less diverse benthic organisms than in a comparable natural water body (Rasmussen 1988).
- If this were the case, the influence of the benthic community on exchanges between the ‘sediments’ and the overlying water column (bioirrigation; bioturbation) would also be less important than in a natural water body.

In developing and applying a protocol for the (bio)monitoring of SADs (section 8), there will be a need to consider reference sites (i.e., natural water bodies with similar constraints in depth, hydrology and irradiation). Both natural and mine-made water bodies will exhibit a range of conditions but in the post-closure period, when the inputs of process and treatment chemicals from the mill will have ceased, there should be at least partial overlap between the two ranges. Nevertheless, as should be clear from the bullet points listed above, the identification of an appropriate reference site will not be straightforward. Small differences between sites can lead to large changes in aquatic communities and conditions. However, as is explained more fully in Section 8, reference sites are largely invoked here not to compare the ‘biological community’ within the SAD facility with the community at a reference site, but rather to find sites where the same biomonitor species are present and can be compared in terms of metal accumulation, metal partitioning, and presence/absence of metal-induced effects.

7.3 Review of data available on post-closure behaviour of subaqueous disposal facilities (MEND)

Based on an analysis of the information provided by NRCan-CanmetMINING, and follow-up reading of related material available in the open literature, underwater disposal of reactive (sulphide-rich) mine tailings has been successful in suppressing sulphide oxidation, preventing the development of ARD and greatly reducing the release of metals.[25] Where underwater disposal of mine tailings has been less successful (i.e., where remobilization of metal cations has been environmentally significant), water

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[25] The review also proved useful as a reminder of the differences between an operating mine, where operational decisions often have to be made within a limited time frame, and laboratory or field experiments. It also served as a helpful reminder that other materials present in smaller quantities are often co-deposited with tailings in SAD facilities.

quality degradation has been linked to situations where (i) the tailings are partially (Paktunc et al. 2011) or periodically exposed to the atmosphere due to water level changes or (ii) tailings have been flooded after an earlier extended period of sub-aerial exposure (Martin et al. 2001). In other cases where metal cation mobilization has been observed, the mechanism responsible has not been sulphide oxidation *per se* but rather one of the following phenomena:

- a decrease in ambient pH (e.g., due to oxidation of NH_4^+, present at elevated concentrations due to the biodegradation of cyanide, Equity Silver) – Price and Aziz (2012);
- dissolution of metal forms (e.g., Cu hydroxide) that had been deposited in the SAD facility during the mine operation phase (treatment sludge, East Lake – Martin et al. (2003b); precipitates from acidic drainage discharged into the impoundment and subsequently neutralized, Equity Silver tailings pond – Lorax (1999));
- alterations to redox conditions at the tailings-water interface have resulted in the dissolution of redox-sensitive precipitates (e.g., radium-bearing barite) formed as part of ore processing or effluent treatment (Martin et al. 2003a);
- remineralization of sedimented plankton (Zn) – Martin et al. (2001).

In other words, the field data available for metal cations in subaqueous disposal facilities are consistent with the summary presented in Section 4 (in particular in section 4.4.3).

The situation for As, Mo and Sb is not unlike that for the cationic metals in that their source is oxidized material present in the tailings due to prior oxidation of the tailings or to co-deposition of some other oxidized material (e.g., elevated As in process water). There are several documented cases of oxyanion mobilization, notably for arsenic (Martin and Pedersen 2002; Martin and Pedersen 2004), that have been linked to changes in redox conditions in the upper tailings strata (e.g., reductive dissolution of Fe oxyhydroxides and release of As close to the tailings-water interface). There is also a reference to Mo and Cu being 'mobile' at the Highland Valley Copper (HVC) site (Larratt 2017); possible sources of Cu and Mo include the continual leaching and periodic flooding of adjacent subaerial tailings, as well as sources other than the subaerial tailings such as a natural creek that drains a mineralized area upgradient from the mine.

The material provided by NRCan-CanmetMINING was also searched for biological information, but there proved to be a dearth of biomonitoring data for SAD facilities. Indeed, there is a marked lack of biological data in general, with the notable exception of the two BC-MEND ML/ARD presentations on the Highland Valley Copper (HVC) site in British Columbia (Larratt and Ohata 2013; Larratt 2017), which document the progressive colonization of the four HVC ponds (microorganisms, periphyton, aquatic plants, fish) and also provide some metal concentrations (Cu, Mo) for the developing biota (riparian vegetation, aquatic plants, benthic invertebrates). In general these concentrations have tended to decrease over time, but few control samples from unaffected habitats are mentioned.

The presentation by Martin et al. (2013) on the recovery of Balmer Lake, ON, includes a slide showing the evolution of the fish community: 1978 no fish → 1996 forage fish → 1999 walleye introduced → 2000 white suckers → 2005 juvenile walleye (i.e., reproducing) → 2008 northern pike. The authors refer to some "impairment of the benthic community" but no supporting data are presented. The only other Canadian BC-MEND ML/ARD presentation to mention biological data was that of Hamblin (2011), who mentioned that stream and vegetation surveys had been carried out in 2002 at the Samatosum Mine site in British Columbia (slide 9), but no data were presented. A common feature of these three Canadian studies (Highland Valley Copper ponds; Balmer Lake; Samatosum Mine) is that there is no mention of sampling the same biological species at control sites (i.e., at sites unaffected by mining activities), for comparison purposes[26].

Finally, the paper by Khozhina and Sherriff (2008) initially looked promising, in that they had collected emergent aquatic plants, some water beetles and one fish species from a subaqueous storage pond, not in Canada and not mine tailings, but at the Salair ore-refining plant (SORP) in Russia. However, there were adjacent subaerial ‘oil tailings’ and the authors report no quality assurance/quality control data (e.g., no blanks, no certified standards, no replicate measurements) and the Pb concentrations in particular are unusually high, casting doubt on their work.

7.4 Colonization of subaqueous environments

Whenever new aquatic habitat is created, for example when a river or stream is dammed and a reservoir is created, its colonization will occur progressively under the influence of both geochemical and biological factors. If the habitat is unfavourable to colonization (see below), the initial phase may occur slowly and then may be followed by a marked acceleration. Colonizing species may arrive from a great variety of sources, for example: in runoff from the catchment, in litter-fall from riparian terrestrial vegetation, as airborne seeds and particulates, or in excrement from birds and terrestrial animals. Even if these propagules are present, colonization may be delayed until some physical or chemical threshold is reached, such as an increase in an organic or nutrient concentration that was limiting species growth. In the colonization of such ‘virgin’ habitats, it has been suggested that early colonizers may gain an advantage and for a period of time be able to out-compete subsequent arrivals, thus delaying the establishment of communities similar to those found at reference sites (Szkokan-Emilson et al. 2010).

In a natural setting, in the absence of metal contamination, the spatial distribution and the abundance of burrowing invertebrates are known to be highly dependent on sediment properties such as particle size distribution and organic-matter content (Wetzel 2001a; Kalff 2002b). The initial benthic colonization of newly flooded terrestrial soils normally occurs rapidly. For example, Paterson and Fernando (1969) studied the colonization of a shallow reservoir (Laurel Creek Reservoir, near Waterloo, Ontario), and demonstrated that colonization of the marginal zones was dominated by species inputs from the creek (i.e., facultative species that had maintained populations in Laurel Creek before the flooding) and that it was virtually complete during the first summer period following dam closure. In a larger impoundment, such colonization would be expected to be somewhat slower (e.g., 3-5 years when a new water supply reservoir is flooded in British Columbia – H. Larratt, personal communication, 2018-05).

Colonization by aquatic plants in lakes is strongly influenced by water transparency, underwater slope and by exposure to waves (Kalff 2002a). Duarte and Kalff (1990) studied the relative contributions of

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[26] In the case of Balmer Lake, control samples were collected at control sites as part of the Canadian EEM programme, as applied to the Red Lake Gold Mines (Minnow Environmental, 2009). Similarly, aquatic and riparian control samples are collected every two years at the Highland Valley Copper facility (H. Larratt – personal communication, 2018-05).

geomorphological characteristics (e.g., depth, littoral slope, exposure to waves) and water quality parameters (total phosphorus concentration, conductivity, and transparency) to the submerged macrophyte biomass in 25 North American lakes. Plant biomass increased with increasing alkalinity and light levels and decreased with increasing slope and wave exposure (i.e., fetch). Rooted aquatic plant growth can also be inhibited by algae blooms (shading).

As mentioned in the Introduction (Section 1), the elevated concentrations of metals and oxyanions present in submerged tailings could potentially affect the rate of colonization of the subaqueous disposal site and also influence which taxa succeed in occupying the habitat (Section 7.4.1). Alternatively, the physical properties of the tailings (e.g., granulometry, cohesion, presence of hardpan or cemented layers), a scarcity of certain key components (e.g., inorganic nutrients; natural organic matter) or other factors (e.g., pH) might also impede colonization and limit the development of the aquatic community. These 'non-metallic' factors are discussed in Section 7.4.2.

7.4.1 Influence of metal concentrations

In the virtual absence of data on the colonization of SAD facilities (with the exception of Highland Valley Copper ponds, Balmer Lake and Samatosum Mine, as mentioned earlier), the scientific literature has been searched for reports of the effects of sediment-associated metals in general on the colonization of wetlands, ponds and the littoral regions of lakes.

The potential role of metals in inhibiting colonization has been explored in field experiments involving the introduction of metal-contaminated sediments into an uncontaminated habitat. Usually uncontaminated sediments are sieved to remove the existing benthic organisms, spiked with dissolved metals, allowed to equilibrate and then introduced back into a natural sediment environment in shallow containers (e.g., trays). The trays are left in place for months to years and sampled over time to determine the rate of colonization and the nature of the colonizing animals. Samples are collected from the adjacent natural sediment at the same time, and the benthic communities are compared in terms of species abundance, richness and diversity. Notable differences between spiked and unspiked sediments are sometimes compared to what is known about the relative metal-sensitivity of the various species (cf. Section 5). Liber et al. (2007) provide a useful summary of this approach in their review of '*In situ*-based effects measures'[27] and Schlekat et al. (2016) describe the artifacts that have to be minimized when one attempts to 'spike' a sediment sample. Examples of this approach include the following studies: Cd (Hare and Shooner 1995; Warren et al. 1998); Cu (Gardham et al. 2014); Ni (Nguyen et al. 2011; Schlekat et al. 2016); Zn (Liber et al. 1996; Burton et al. 2005). Several representative studies are described below.

- One of the earlier examples of this approach is the study by Hare and Shooner (1995), who followed the *in situ* colonization of spiked lake sediments that differed only in their concentration of Cd (≈ 0.007 (control, no Cd added), 0.05, 0.17, 0.54, 2.7 µmol/g dry weight). The spiked sediments were returned to the lake bottom by divers and left there for 14 months. On recovery, the sediments were sieved and the resident insect larvae were identified, enumerated and analyzed for Cd. The experimental design ensured that Cd concentrations in the water overlying the sediments were uniform (and low), whereas the Cd concentrations in the sediment

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[27] See the section entitled '*Design considerations for sediment colonization experiments*'.

pore water increased by a factor of 10^3 from the control sediment to the most contaminated trays. For the chironomids *Procladius* (*Holotanypus*) sp. and *Sergentia coracina*, two of the more abundant colonizing species, their population densities were unrelated to the Cd gradient, even though both taxa accumulated Cd in direct relation to its concentration in the sediment pore water and the host sediment. Cadmium concentrations in *Chironomus* (*salinarius* gp.) sp. larvae also increased as a function of the sediment Cd gradient, but in this case larval abundance was negatively correlated with the sediment Cd gradient. In parallel laboratory experiments, metal-naïve larvae were offered a choice of sediments that differed in Cd concentrations; the *Chironomus* (*salinarius* gp.) sp. larvae did not avoid the high-Cd sediment, suggesting that the lower abundance of this species in the field experiment was likely a result of metal toxicity. However, it should be noted that this effect was only seen at the highest Cd concentration, where the Cd concentration in the interstitial water was ≈ 150 µM.

- In a follow-up experiment, carried out with the same approach but in a different lake, Warren et al. (1998) again spiked a natural lake sediment with Cd, returned the sediments to the lake bottom in trays to allow them to become colonized by the indigenous benthic organisms. They recovered the sediments after 11 months and characterized the benthic communities in each sediment tray and in the adjacent lake sediments. The spiked sediments represented a 38-fold increase in total Cd concentrations (0.02 (control), 1.5, 3.0, 4.5, 6.0, and 7.5 µmol Cd/g dry weight) and the [Cd]/[AVS] ratio varied from <0.01 in the control to 2.0 in the most contaminated sediment (see Sections 4.1 and 4.3 for a description of the SEM – AVS approach). Although the spiked Cd concentrations were considerably higher than those found even in severely contaminated lake sediments, there was no effect of Cd on the mean community density (individuals/m^2). At the population level, no taxon disappeared in response to the Cd treatment and the densities of most individual taxa appeared to be unaffected by the Cd gradient.
- In a similar experiment, Liber et al. (1996) collected an uncontaminated pond sediment, spiked it with five Zn concentrations (yielding SEM concentrations, predominantly Zn, of 0.8, 1.5, 3.0, 6.0, and 12.0 µmol/g dry weight) and returned the sediments to the pond bottom, within an enclosed area isolated from the rest of the pond. Samples were collected on five dates over a period of 12 months and subjected to both chemical analysis and macroinvertebrate enumeration. The measured AVS concentrations consistently exceeded the SEM concentrations, except in the most contaminated sediment, and Zn in the pore water was never present at concentrations likely to pose a threat to benthic macroinvertebrates (Liber et al. 1996). Not surprisingly given these geochemical results, the colonization of the Zn-spiked sediments did not differ from that of the control sediments; macroinvertebrate abundances in the sediment trays were similar to or greater than abundances in the control sediments on all sampling dates.
- In a final example of the spiked-sediment approach, but this time with Ni, Nguyen et al. (2011) compared the benthic communities in their sediment trays with the community in the adjacent lake sediments. Provided that the (SEM – AVS) values were ≤ 0.4 µmol/g, there were no significant differences between the Ni-spiked sediments and the native communities, but for values >2 µmol/g clear adverse effects were observed.

To summarize the results of these experiments, metal effects on the colonization of sediments were only noted at very high metal concentrations and under conditions where the extractable metal concentrations (SEM) were well above the acid volatile sulphide (AVS) concentration. As pointed out by Hare and Shooner (1995), when the population density of a particular benthic invertebrate decreases under such conditions, the effect could in principle reflect either metal-induced toxicity or avoidance behaviour on the part of the animal.

7.4.2 Influence of chemical and physical factors other than metals

In addition to the 'metal' effects considered to this point, other chemical and physical factors may affect the colonization of a SAD facility. At the base of the food chain, primary producers such as phytoplankton or macrophytes require inorganic nitrogen and phosphorus, with the latter (i.e., phosphate) being the limiting nutrient in most freshwater habitats (Kalff 2002a). In a SAD facility the tailings will normally be phosphate-poor, and in addition iron oxyhydroxides formed at the tailings-water interface by the oxidation of iron sulphides will tend to act as a sink for any phosphate that is present and limit its phytoavailability. Given these considerations, selective fertilization may be considered as part of the protocol for establishing and maintaining a SAD facility.

Microbes and benthic invertebrates require organic matter as an energy source. Normally this is provided by transport from the watershed (allochthonous sources) and by primary production within the water body (autochthonous sources). Immediately after the water cover is established, the development of the benthic community will be limited by a shortage of labile organic matter and this shortage will only be alleviated when the annual cycle of phytoplankton and plant growth, death and sedimentation is well established and an organic layer has accumulated on the sediment surface.

The physical nature of the submerged tailings may also affect their suitability for benthic colonization. Epibenthic invertebrates feed on the tailings/sediment surface and their ingestion of the solid substrate will be a function of the size and cohesion of the sediment particles and of the gape of their mouth. Endobenthic animals create their own microenvironment by burrowing into the substrate, and in their case the suitability of the submerged tailings will also be a function of the cohesion of the substrate, which will determine if burrows can be created and if they are stable thereafter.

Crushing and grinding during mill operations may result in sharp particles, the presence of which would be expected to deter ingestion or tunnelling activities. For example, in avoidance studies with earthworms and soils prepared with varying proportions of a natural loam and an OECD standard artificial soil, Brami et al. (2017) reported that the proportion and grain size of the sand component were the key factors that influenced the tendency of the earthworms to prefer one soil over another.

Also of importance as a physical factor is the possible formation of 'hardpan' layers, hardpan being defined as "cemented layers of secondary (oxy)hydroxide and (hydroxy)sulfate minerals" (Lindsay et al. 2015). These layers are formed when the products of sulphide oxidation precipitate as amorphous solids in the void space of the tailings and cement the particles together. Secondary (oxy)hydroxide and (hydroxy)sulfate formation and creation of a cemented layer would be accelerated by subaerial weathering prior to flooding. Hardpan may limit pore-water movement within the tailings (advection), impede oxygen diffusion into the tailings, and also act as a (temporary) sink for other metals and oxyanions that are released during the weathering of sulphidic tailings (Graupner et al. 2007; Kohfahl et al. 2010; Quispe et al. 2013).

In their review article, Lindsay et al. (2015) focused on subaerial tailings and did not explicitly mention any effects of hardpan formation on the colonization of the tailings. As mentioned above, hardpan formation is linked to the oxidation of sulphidic iron-rich tailings and normally the hardpan is found close to the oxidized zone, where the products of sulphide oxidation collect. In tailings that are exposed to the atmosphere (and in soils), hardpan layers tend to form at depth, in the zone where the products of sulphide oxidation collect as water migrates downward (Graupner et al. 2007; Lindsay et al. 2015). Hardpan could conceivably be formed at the surface of submerged tailings, especially if the tailings have a high sulphide content, but no examples of this phenomenon could be found in the geochemical literature. If colonization of the submerged tailings by aquatic plants and benthic invertebrates occurred early enough, the developing biological community might well impede the formation of a 'cemented' layer at the tailings surface.

A bibliographic Internet search (*Web of Science*) using 'hardpan' and 'colonization' as keywords returned zero 'hits'. However, anecdotal evidence from Lac Aux Dorés, a lake near Chibougamau in north-central Quebec, does suggest that the existence of hardpan at the tailings surface can severely limit the colonization of submerged tailings. The dike retaining a tailings pond adjacent to Lac aux Dorés was breached in 1976 and 1984, and an undetermined but large amount of sulphide-rich tailings escaped into the lake; the area affected by the tailings spill is still visible from the (repaired) dike. In 2008, samples of the surficial sediments and the sediment-dwelling invertebrates were collected from various locations in the lake (Proulx and Hare 2009). The field team found that there had been no little or no colonization of the submerged tailings, despite the lengthy submersion, and their attempts to take grab samples of the substrate were thwarted by the hardpan that had formed on the surface of the tailings. In their report, Proulx and Hare (2009) tentatively attributed the absence of benthos to the presence of the hardpan and the absence of natural organic matter. Negligible sedimentation had occurred on top of the tailings, probably because of wave action that had kept the hardpan surface 'clean'. As mentioned earlier, benthic animals normally prefer muddy sediments (i) that are of a texture allowing them to create their burrows, and (ii) that contain labile natural organic matter at concentrations sufficient to serve as a food source.

Finally, in SADs with shallow (<1 m) water covers, ice scouring represents another physical factor that could affect the recolonization of benthic macrophytes, and possibly benthos. This was postulated as a control governing the distribution of *Chara* sp. at the Quirke Mine (Martin et al. 2003a).

7.5 Reclamation of SAD facilities

Establishment of aquatic ecosystems will occur naturally in a SAD facility but can be accelerated by fertilizing with phosphorus to produce algal blooms and by introducing suitable substrate or aquatic plants, in part to accelerate colonization by desirable aquatic life. This section is largely based on methods used by Highland Valley Copper and guidance provided by Heather Larratt reported in Larratt and Ohata (2013).

Highland Valley Copper used a variety of methods to accelerate colonization by bacteria, periphyton and macrophytes. Aquatic plants were introduced with a root frame. The plants subsequently spread and the frames corroded rapidly (within 10 years) removing the danger of wildlife entanglement. Other sources of introduced species included sedge and rush transplants, cattail transplants or seed, riparian grass seed mix or fish from a hatchery. Backhoes were used to chop salvaged sedge transplants into 20 x 20 cm chunks suitable for planting. Fish should be acclimatized before introduction and may need a spawning channel to sustain the population.

Caution is merited here, since it is illegal to transport benthic invertebrates without a permit. Care must be exercised to avoid the accidental introduction of aquatic invasive species. Methods for introducing zooplankton and invertebrates include selective techniques with a screened bucket and non-selective approaches with soaked hay bales or introduced plants.

One of the main factors determining biological productivity and lake chemistry is the rate of carbon production associated with plankton and aquatic plants (macrophytes). Phytoplankton are a major source of food for zooplankton and benthic invertebrates, both of which serve as fish food. Bacterial flora expand rapidly once algae and plant organic carbon are available. Primary production in freshwater systems is typically limited by available phosphorus and phosphorus may be added as a form of bioremediation to create desired levels of productivity.

With adequate water chemistry and inorganic nutrients, photosynthetic bacteria and chemotrophs may form a nutrient/vitamin-rich organic sediment layer called periphyton, which consists of a complex biofilm of algae, cyanobacteria, heterotrophic microbes and detritus that attach to submerged surfaces. The upper few mm of organic sediments and biofilms host the vast majority of aquatic bacteria and this layer is crucial habitat for benthic invertebrates that are food for fish.

SAD facilities are most effective at removing dissolved metals if there is a constant supply of biologically and chemically active particulates sinking through the water column. The primary mechanism of metal removal in many lakes is adsorption of metals (e.g., Cu, Pb, Zn, Cd and Ni) onto surfaces of algae and bacteria (Wetzel, 1975). The aging microflora and adsorbed metals settle to the bottom and form sediments. Another removal mechanism for metal ions, although relatively minor by comparison, is by microflora absorbing nutrients (e.g., Cu, Zn, Co) or mistakenly absorbing non-nutrient elements (e.g., Cd, As). Aquatic macrophytes, filamentous green algae and biological debris can offer a massive surface area for metal adsorption. They also attenuate wave energy, which results in decreased sediment re-suspension.

As is discussed in Section 8, post-reclamation monitoring should include the chemistry of the water cover and tissue metal concentrations in flora and fauna to check for bioaccumulation at different levels of the food chain. It is important to monitor all water inputs to a flooded impoundment to ensure changes in water chemistry can be predicted and traced back to the source.

8 Summary and Recommendations

8.1 Recapitulation

The preceding literature review has focused on the biogeochemistry of selected metals and oxyanions in contaminated freshwater sediments and in submerged mine tailings. As a starting point, the behaviour of metals and oxyanions in the overlying water column and in the underlying solid phase (sediments/tailings) was considered, followed by an analysis of their interactions with the biota that had colonized these metal-rich sites. This information was derived largely from laboratory studies, from work done on metal-contaminated lakes/ponds, and from post-closure studies of subaqueous disposal (SAD) facilities at mines in Canada and elsewhere.

With respect to this latter source of information, many of the studies of tailings diagenesis identified in Section 7 were limited spatially and temporally. In addition, the investigations of metal/oxyanion release were often carried out at sites where the tailings had been partially oxidized, either during processing or during an initial period of subaerial exposure, before flooding. Nevertheless, with few exceptions it is clear that subaqueous disposal has met the primary objectives of limiting sulphide oxidation to within a few millimetres or centimetres of the sediment-water interface and minimizing metal/oxyanion release. Where tailings predicted to be acid generating under aerial conditions were flooded prior to significant oxidation, post-closure metal/oxyanion levels in SAD facilities in British Columbia meet discharge criteria set by the Ministry of Environment without additional mitigation or drainage treatment (e.g., Samatosum, Kemess and Huckleberry Mines)..This success, coupled with an apparent lack of attention to aquatic reclamation, has however resulted in limited post-closure monitoring and study of tailings diagenesis and biological colonization.

The present section draws upon this information and provides guidance regarding recommended tools and methodologies that could be used to predict and/or monitor the biological and ecological effects of submerged tailings. This section is not designed to be a detailed guide to field or laboratory work, but rather should be viewed as a general outline of how to approach the challenge of assessing the possible biological effects of the subaqueous disposal of mine tailings. Given the paucity of biological data from existing SAD facilities, some exploratory sampling and analysis will undoubtedly be necessary.

8.2 General principles for the geochemical and biological monitoring of Subaqueous Disposal Facilities

The approach to the assessment of SAD facilities must include coordinated geochemical and biological measurements, and should include measurements within the SAD facility and, where possible, in one or more reference areas that harbour the same biomonitor species[28]. The number of sampling sites within the SAD facility should reflect its size and heterogeneity (starting with a large sample size and reducing it if spatial variability proves to be low). The frequency of the measurements at these sites should reflect the anticipated temporal variability, both short-term (diurnal, seasonal) and long-term (annual, decadal).

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[28] The need for reference sites and the challenge that this represents are discussed in Section 8.5.

Sampling is usually focused on the active growing season but some winter measurements may be necessary if under-ice depletion of dissolved oxygen is suspected.

To the extent possible, the same measurements and the same methodologies should be used in different SAD installations, to facilitate comparisons among the facilities. In this context, the technical guidance issued for 'Environmental Effects Monitoring' for metal mining (Environment Canada 2012), as well as the earlier reports issued by the Aquatic Effects Technology Evaluation Program in the late 1990s (AETE 1999b) constitute a valuable source of Canadian expertise in this area, particularly for biological measurements.

At the same time, the approach should also be reasonably flexible so that it can consider the historical data that are available at the site and can be adapted to the specific characteristics of the SAD facility. Similarly, both for cost effectiveness and in the context of site-specificity, a phased approach is warranted, starting with a general reconnaissance (physical, chemical, biological) step and then focusing on areas of concern and data gaps. For example, if the initial reconnaissance and monitoring data reveal a biologically impoverished impoundment but no geochemical limitations, then the initial phase might target the physical properties of the submerged tailings (e.g., grain size, cohesion, penetrability), to determine if the relative lack of biological colonization is due to a physical impediment. Conversely, if the impoundment contains what appears to be a diverse and abundant biological community, then the initial phase might focus more on determining the 'health' of this community, using a passive biomonitoring approach as described in Sections 4.5.1, 8.4 and Appendix E. Such a step-by-step approach was adopted for the Environmental Effects Monitoring programme for metal mining and it is entirely compatible with current theory and practice in the field of 'adaptive monitoring' (Lindenmayer and Likens 2009; Arciszewski et al. 2017).

Finally, some of the measurements that are proposed in this section have been shown to be useful in freshwater environments (lakes, streams) with metal-contaminated sediments, but they have not been applied in SAD facilities. Examples include Teflon strips to collect authigenic phases, penetrometers to determine the mechanical resistance of tailings to benthic colonization and caged invertebrates to act as active biomonitors). Small-scale studies of these methods are needed to evaluate their applicability as recommended monitoring techniques for submerged tailings.

8.3 Geochemical guiding principles

For metals that yield metallic cations in solution, the focus should be on approaches that will yield estimates of the free-metal ion concentration in solution (given the recognized usefulness of the free-metal ion concentration as a predictor of metal bioavailability). Possible analytical approaches are summarized in Section 2.2.6. Measurements should be carried out in the overlying water and in the underlying sediments/tailings.

In the aqueous phase (overlying water):

- Analyses should include the major cations and anions (Ca, Mg, Na, K; Cl, SO_4, CO_3; Fe, Mn, Al), pH and DOC, since all are needed both for speciation calculations and for estimates of metal bioavailability. In addition, concentrations of plant nutrients (NH_4^+, NO_3^-, PO_4^{3-}) are needed to assess possible chemical factors that limit primary production by algae and aquatic plants.

- Trace-metal-clean techniques should be applied to avoid inadvertent contamination of the samples during sampling (Nriagu et al. 1993; Horowitz et al. 1994) and appropriate QA/QC protocols should be applied.
- If the presence of process chemicals is deemed to be likely (e.g., early in the period after closure of the mine, shortly after the discharge of tailings has ceased), then the analyses should include these compounds and their degradation products.

In the sediments/submerged tailings phase:

- The focus should be on the upper strata, i.e., the zone that is in contact with the benthic fauna and flora, and that may be a source or sink for the metals/oxyanions of interest (NRCC 1988). Physical or mechanical measures (penetrometer) can be used to evaluate the suitability of the submerged tailings for burrowing invertebrates (Bokuniewicz et al. 1975; Crane and Merz 2017).
- In the sediments/submerged tailings phase, measurements of the pore-water composition should be carried out at different depths (e.g., with pore-water peepers) to evaluate (i) fluxes across the sediment-water interface, and (ii) biogeochemical processes governing contaminant mobility (Table 4-1).
- Also in the sediments/submerged tailings phase, measurements on the solid phase can be used to evaluate the geochemical controls on pore-water metal/oxyanion concentrations (e.g., SEM – AVS measurements; amorphous iron oxyhydroxides, their associated metals/oxyanions, and pH – see Table 4-2).
- Sediment coring, and associated dating methods, can be used to examine trends in substrate physical and chemical properties, and link these variables to biological recolonization.
- Sediment traps can be installed to determine the amount of material that is settling out of the water column (e.g., phytoplankton; suspended (or resuspended) sediment).

8.4 Biological guiding principles (tiered approach)

- Both passive and active biomonitoring approaches are possible. In the passive approach, indigenous plants and animals that are present in both the SAD facility and in the reference area(s) are located, collected and analysed. In the active biomonitoring approach, metal-naïve biomonitor organisms are chosen, introduced into the SAD facility, and monitored over time.
- In both cases, the focus should be on plants and animals for which (i) there is direct contact with the bottom sediments/tailings (e.g., periphyton, rooted aquatic plants, benthic invertebrates, bottom-feeding fish and waterfowl) and (ii) there are already some biomonitoring data (in Canada) (Tables 4-3, 4-4).
- When feasible (e.g., in multicellular biomonitor organisms), it is preferable to focus on specific organs or tissues (e.g., liver, gills, gonads; roots, stems, leaves) rather than on the whole biomonitor organism. Initial measurements should aim to determine the level of metal/oxyanion bioaccumulation. If metal/oxyanion bioaccumulation in the SAD facility is markedly higher than in the reference area(s), then the subcellular distribution of the metal/oxyanion could be investigated to determine whether the metal/oxyanion had been successfully detoxified (see Section 4.5.2). If metal/oxyanion detoxification is incomplete, biomarkers known to be sensitive to metals/oxyanions could be used to confirm an effect.

- In addition to the biomonitoring approach, the application of techniques designed to evaluate the biodiversity (and its temporal changes) in the SAD facilities and the reference area(s) may also be required (see Section 4.5.4).

Some practical advice concerning the geochemical and biological measurements described above has been collated in Appendix E, along with references to both the present report and to the scientific literature.

8.5 Reference sites

To assess the biological status of a subaqueous disposal facility, it will be important to have identified one or more reference sites (i.e., natural water bodies with similar constraints in depth, hydrology and irradiation). Both natural and mine-made water bodies will exhibit a range of physical and chemical conditions, with notable seasonal variations in both cases; nevertheless, it should be possible to achieve at least partial overlap between the two ranges in the post-closure period, when the inputs of process and treatment chemicals from the mill will have ceased. However, as has been pointed out in Section 7.2, SAD facilities have many characteristics that differ from those of a typical pond or small lake, and consequently the identification of an appropriate reference site will not be straightforward. Indeed, several reviewers of this report raised this point and noted that finding 'good' or 'adequate' aquatic reference sites has been a challenge for a number mines faced with the requirement to carry out environmental effects monitoring (EEM).

Small (physical) differences between sampling sites can lead to large changes in aquatic communities. In an EEM context, these differences can compromise the comparison between the reference site and the downstream site affected by the mine effluent. These differences should be of less importance in the present context, where the focus is on metal bioavailability and not on community structure[29]. Reference sites are largely invoked here not to compare the 'biological community' within the SAD facility with the community at a reference site (as is done in an EEM programme)[30], but rather to find sites where the same biomonitor species are present and where these species can be compared in terms of metal accumulation, metal partitioning, and presence/ absence of metal-induced effects. In other words, the primary need for reference sites (plural) is to provide specimens of the biomonitor organisms that are present in the SAD facility, but which haven't experienced the conditions within the SAD system; these 'control' specimens would be compared to those collected within the SAD facility. A water body would qualify as a reference site if the physical constraints of depth and size are met, if it is demonstrably uncontaminated, and if it contains the desired species. Note that this approach focuses on metals/oxyanions as the principal stressors in the SAD facility; it is not designed to provide evidence of the effects of other stressors, such as an overwinter oxygen deficit.

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[29] It follows that 'survivor bias', or the absence in the SAD facility of a particular species that is present at the reference site, will not be a problem since the biomonitor species in the SAD facility is being compared to same species collected at the reference site(s).

[30] Note that the MMER regulations were amended in May 2018 (and renamed the Metal and Diamond Mining Effluent Regulations). The 2012 EEM guidance document remains current.

8.6 Representative scenarios

As mentioned in Section 8.2, the phased approach used to assess the biological status of a SAD facility will vary from one facility to another. It would be unrealistic to propose a 'one size fits all' protocol, but nevertheless, as a practical guide, an invented scenario is described below. The emphasis is on a phased approach that resembles adaptive monitoring in that there are several potential 'yes/no' branches in the development of the biomonitoring programme (Table 8-1). This table is linked to Appendix E, which contains practical advice about the geochemical and biological measurements recommended for SAD facilities and reference areas.

Table 8-1: Example of how a phased biological assessment of a subaqueous disposal facility might proceed.

Step 1: Compile an inventory of general conditions	
o topographic layout; bathymetry; evidence of wave-induced resuspension of tailings; o visual estimation of the type and spatial distribution of aquatic vegetation; o inventory of previous and ongoing solid and drainage inputs – type, magnitude, composition and time of deposition; o history of mine-related inputs to the SAD facility (material type, distribution); o key climate conditions such as ice-free period, direction of prevailing wind, drainage inputs and outputs; hydraulic residence time, etc.	This generic step would be quasi-universal.
Step 2: Overlying water	
o compile and examine existing data for the quality of the overlying water (dissolved components)[31], both in the SAD facility and at the reference site(s); o identify which metals[32] are potential problems (based on total dissolved metal); o determine if total dissolved concentrations of these metals meet relevant water quality guidelines (not effluent discharge guidelines but rather Canadian Water Quality Guidelines or site-specific water quality guidelines); o if sufficient water quality data are available, run the BLM for each of these metals and determine if various toxicity modifying factors (pH, Ca^{2+}, Mg^{2+}, DOM) are sufficient to avoid exceedance of the guideline; o collect physical profiles of water column (conductivity, temperature, DO) to evaluate mixing properties and the potential for adverse conditions that may limit benthic and/or water column productivity (e.g., seasonal stratification and bottom water oxygen depletion).	The results of this step should indicate whether overlying water quality is problematic or not (for the development of a diverse and productive planktonic community).

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[31] If the necessary data are not available, plan for their acquisition (see Appendix E).

[32] The term 'metal' is used in the table in a generic sense, and includes 'oxyanions'.

Step 3: Submerged tailings (geochemistry)

- compile and examine existing data for the quality of the superficial tailings (0-15 cm), e.g., logs for coring, grab sampling and Teflon strips (grain size, porosity, type and distribution of different types of tailings; mineralogy, weathering and depositional features);
- compile and examine existing data on pore-water profiles (O_2, NO_3^-, SO_4^{2-}, Fe^{2+}, Mn^{2+}, DOC, metals identified in #1, etc.) and analyses of submerged tailings (AVS, SEM; in oxic layer, amorphous iron oxyhydroxides, their associated metals, pH, and particulate organic matter (POM))[32];
- from the pore-water profiles, determine direction of the metal fluxes across the tailings/water interface; if flux is out of the sediment, calculate quantity of metal leaving tailings and entering the water column;
- based on AVS and SEM values, and POM concentration, determine where the surficial tailings fall on the plot shown in Figure 4-2 (upper right or lower left quadrants);
- based on the amorphous iron oxyhydroxides, their associated metals, pH and [POM], estimate the free metal ion concentrations in the oxic pore waters (Table 4-2) and compare these to the CWQG values (expressed as the free metal ion);
- if the presence of process chemicals is deemed to be likely, then the analyses of the pore water and the overlying water should include these compounds and their degradation products.

The results of this step should indicate whether or not the quality of the submerged tailings and their constituent pore water is potentially problematic or not (for the development of a diverse and productive benthic community).

Step 4: Submerged tailings (habitat, biology)

- look for evidence of constraints or disturbance such as low dissolved oxygen concentrations, excessive wave action, or presence of hardpan layers;
- compile and examine the existing data on physical nature of the tailings (grain size; porosity; penetrability)[32];
- building on what was uncovered in step #1 (estimation of the type and spatial distribution of aquatic vegetation), compile and examine the existing data regarding the nature, location and abundance of the indigenous biota in the SAD facility (submerged aquatic plants; emergent aquatic plants; benthic invertebrates; forage fish; migratory waterfowl; …)[32];
- consider the use of echo-sounders or drones equipped with multispectral cameras to determine the extent of submerged vegetation;
- identify potential biomonitor species and verify their presence in the reference area(s).

The results of this step will be compared with the predictions of Steps 2 and 3.

Step 5: Integration of the results of Steps 1→4 and planning of acquisition of new data as needed

- compare predictions of Steps 2 and 3 with the observations from Step 4;
- if the initial reconnaissance reveals a visibly biologically impoverished impoundment, then go to Step 6a;
- if the impoundment contains what appears to be a diverse and abundant biological community, then go to step 6b;
- refine the list of constituents of interest and metals of concern.

Note the divergence of paths here.

Step 6a: Biologically impoverished impoundment

- evaluate the physical properties of the impoundment (e.g., wave action, fluctuations in water level) and the mechanical properties of the submerged tailings (e.g., grain size, cohesion, penetrability), to determine if the relative lack of biological colonization is due to a physical impediment;
- evaluate the biogeochemical properties of the water column (water quality, seasonal distribution of dissolved oxygen) and substrate (chemical composition of solids and pore water) to determine if the relative lack of biological colonization is due to a chemical impediment;
- use active biomonitoring (i.e., transplantation of biomonitor species into the SAD facility) to determine if the biomonitor survives in the presence of the submerged tailings.

The results of this step should indicate if the impoverishment is due to a lack of colonizing species, or to a problem with their survival.

Step 6b: Well-colonized impoundment

- focus on determining the 'health' of this community, using a passive biomonitoring approach as described in Section 4.5.1 and outlined in Appendix E;
- biomonitoring would initially include the determination of metal concentrations in biomonitor species established in the SAD facility and present in the reference areas;
- if metal/oxyanion bioaccumulation in the SAD facility is markedly higher than in the reference area(s), then the subcellular distribution of the metal/oxyanion could be investigated to determine whether or not the metal/oxyanion had been successfully detoxified (see Section 4.5.2). If metal/oxyanion detoxification is incomplete, biomarkers known to be sensitive to metals/oxyanions could be used to confirm an effect.

The timing of the various steps outlined in Table 8-1 will depend on the situation within the SAD facility (Is it still in operation? If not, for how long has it been closed?). There may be an opportunity to perform some field trials and preliminary measurements before mine closure. This field trial approach was used at the Louvicourt SAD facility and is commonly used in terrestrial reclamation.

8.7 Data and knowledge gaps:

During the preparation of the present report and during its review by the MEND steering committee, a number of 'data and knowledge gaps' were identified. Given that this information could be helpful in guiding future studies of subaqueous disposal facilities, we have compiled a list of the main 'gaps' that were identified.

- There is a general lack of information about the hydrobiology of SAD facilities, their inherent heterogeneity, possible impediments to becoming productive habitat, and their variability over time (diagenetic changes) . To address this situation, a limited campaign of field measurements could be carried out in a representative selection of SAD facilities:
  - emphasis on mine-made facilities, not natural lakes;
  - emphasis on sites where the tailings were not exposed sub-aerially before flooding or on the edge of the facility;
  - emphasis on sites where disposal of mine tailings ceased at least 5 years ago.

This information could then inform future decisions about the monitoring of SAD facilities and the degree to which detailed investigation of the distribution and productivity of the resident biota is necessary. The information would also feed into an 'adaptive monitoring' approach and guide decisions about how to phase future work.

- Field trials should be carried out with a number of the monitoring techniques that have been recommended (Sections 8.2, 8.3, 8.4 and Appendix E). These techniques have been shown to be useful in metal-contaminated lakes, but they haven't been field-tested on submerged tailings.

  Examples of such techniques include the following:
  - penetrometers to determine the mechanical resistance of tailings to benthic colonization;
  - Teflon strips to collect authigenic sediment phases;
  - methods for measuring changes over time in important factors, such as organic content, depth of oxygen ingress, sediment accumulation;
  - best ways to take cores and to obtain pore-water profiles
  - SEM – AVS model (equation 4-2);
  - equilibrium adsorption (surface complexation) model (Table 4-2);
  - caged invertebrates to act as active biomonitors;
  - e-DNA (DNA Barcoding) to evaluate macroinvertebrate diversity (section 4.5.4).
  - use of sensors in monitoring water column and sediment (e.g., dissolved oxygen, nitrate, ammonium, sulphate);
  - use of drones to provide inventory of general conditions (e.g., topographic layout; bathymetry; evidence of wave-induced resuspension of tailings; visual estimation of the type and spatial distribution of aquatic vegetation; drainage inputs).
- Process chemicals (e.g., cyanide; xanthates) and thiosalts have been detected in active SAD facilities (i.e., in the period before mine closure), but little is known about the possible persistence of these substances once active disposal of mine tailings has ceased. Measurements could be carried out in tailings pore water and in the overlying water column.
- Criteria are needed for defining successful reclamation of SAD facilities in different climatic regions and with different limnological constraints (e.g., suboxic water cover during winter). In considering the overall objective of a typical mine closure/reclamation plan (e.g., to enable the establishment of an 'adequately healthy, productive ecosystem – including no negative impacts to adjacent ecosystems'), there is a need to define 'adequately healthy and productive' and to consider both ecosystem function (productivity) and ecosystem structure (valued species). This knowledge gap is linked to the need to update Table 4-6 in the present document.

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10 Appendices

Appendix A:

- Summary of known exceptions to the Biotic Ligand Model
- Examples of the influence of natural dissolved organic matter (DOM) on trace metal bioavailability

Appendix B:

- Compilation of successful applications of the equilibrium adsorption (surface complexation) model to oxic sediments

Appendix C:

- Examples of Species Sensitivity Distribution (SSD) plots for the chronic toxicity of individual metals

Appendix D:

- List of Reports, conference proceedings and BC-MEND ML/ARD slide presentations provided by NRCan-CanmetMINING

Appendix E:

- Practical advice regarding geochemical and biological measurements recommended for SAD facilities and reference areas (Section 8).

10.1 Appendix A (Summary of the known limitations to the BLM approach)

Table A- 1: Summary of the known limitations to the BLM approach

Limitation[1]	Examples	Comments	
- non-competitive inhibition by H^+*	Chowdhury and Blust (2001); François et al. (2007)	- influence of pH may not be correctly handled by BLM	
- direct influence of NOM on organism response	Parent et al. (1996); Wood et al. (2011)	- role of NOM may not be limited to its ability to complex metals	
- boundary layer chemistry	Playle and Wood (1989); Liu et al. (2017a)	- chemistry of the boundary layer next to the biological surface may differ from that of the bulk solution	
- assimilable LM complexes (mechanism 2, Figure1)	Errécalde and Campbell (2000); Fortin and Campbell (2001)	- in the presence of LM complexes, where L is an assimilable metabolite, M bioavailability ↑	
- lipophilic ML_n^0 complexes* (mechanism 3, Figure1)	Turner and Mawji (2005); Boullemant et al. (2009)	- in the presence of neutral, lipophilic M-L complexes, M bioavailability ↑	
- diffusion of M from bulk solution to the biological interface is rate-limiting	Fortin and Campbell (2000); Hudson (2005); (Degryse et al. 2012)	- a key assumption underpinning the BLM is not met, meaning BLM inapplicable, but few such examples	
- trivalent metals	Wilkinson et al. (1990); Crémazy et al. (2013); (2014)	- trivalent metals relatively under-studied, and exceptions more frequent than for divalent metals	
- diet-borne metals ignored*	Fisher and Hook (2002); Meyer et al. (2005); Wang (2013a)	- BLM may underestimate the toxicity of metals that are largely taken up from food	
- acclimation*	Muyssen and Janssen (2004); Niyogi et al. (2004)	- chronically exposed organisms will tend to acclimate to and become more tolerant of metals, meaning BLM may overestimate toxicity	

[1] Limitations mentioned in the earlier reports (MEND 1993, 2009) are indicated by an asterisk*. Additional examples can be found in the following references: Campbell (1995); Campbell et al. (2002); Paquin et al. (2002); Hassler et al. (2004); Slaveykova and Wilkinson (2005); Campbell and Fortin (2013); Zhao et al. (2016).

Table A-2: Influence of natural dissolved organic matter (DOM) on trace metal bioavailability.

A – BLM behaviour, metal as available as expected

Species	Metal	Response	Reference
1. *Pseudokirchnereilla subcapitata* *Chlamydomonas reinhardtii* (freshwater algae)	Cd	metal uptake metal uptake	Vigneault & Campbell (2005)
2. *Pseudokirchnereilla subcapitata* (freshwater alga)	Cd	metal uptake	Verheyen et al. (2014)
3. *Chlamydomonas reinhardtii* (freshwater alga)	Cd, Ni, Pb		Kola & Wilkinson (2005); Worms et al. (2007, 2015)
4. *Chlorella kesslerii* (freshwater alga)	Cd	metal uptake	Bayen et al. (2006)
5. *Chlorella kesslerii* (freshwater alga)	Cd, Cu	metal uptake	Lamelas & Slaveykova (2007)
6. *Chlorella kesslerii* (freshwater alga)	Cd, Ni	metal uptake	Worms et al. (2010, 2007)
7. *Monochrysis lutheri* (euryhaline alga)	Cu	growth inhibition	Sunda & Lewis (1978)
8. *Chlorella salina* (marine alga)	Cd,Cu Pb, Ni	metal uptake	Slaveykova et al. (2009b)
9. *Thalassiosira weissflogii* (marine alga – diatom)	Cu, Pb	metal uptake	Sánchez-Marín et al. (2010)
10. *Daphnia magna* (freshwater cladoceran)	Cu	mortality	Meador (1991)
11. *Ceriodaphnia silvestrii* (freshwater cladoceran)	Cu	toxicity (mortality)	Santos et al. (2008)
12. *Gammarus pulex* (freshwater amphipod)	Cd	metal uptake	Pellet et al. (2009)
13. *Hyalella azteca* (freshwater amphipod)	Ni	metal uptake	Doig & Liber (2006)
14. *Brachionus plicatilis* (marine rotifer)	Cu	toxicity (mortality)	Tait et al. (2016)
15. *Xenopus laevis* (embryo) (freshwater amphibian)	Cu	larval development	Buchwalter *et al.* (1996)
16. *Cyprinis carpio* (common carp)	Cu	metal uptake (gill and whole fish)	Van Ginneken *et al.* (2001)
17. Marine bacterium (Gram negative)	Cu	glucose uptake	Sunda & Gillespie (1979)
18. *Sinorhizobium meliloti* (Gram negative bacterium)	Cd	metal uptake	Slaveykova et al. (2009a)
19. *Mytilus edulis* (marine bivalve)	Cu	metal uptake (gill)	Lorenzo *et al.* (2005)
20. *Mytilus edulis* (marine bivalve)	Pb	metal uptake (gill)	Sánchez-Marín et al. (2011)

21. *Paracentrotus lividus* (sea urchin larvae)	Cu	toxicity (embryo-larval growth)	Lorenzo *et al.* (2006)
22. *Crassostrea gigas* (American oyster embryos)	Cu	toxicity	Brooks et al. (2007)
23. *Hydroides elegans* (marine polychaete)	Cu	larval development	Qiu et al. (2007)

B – non-BLM behaviour, metal more available than expected

Species	Metal	Response	Reference
1. *Chlorella kesslerii* (freshwater alga)	Pb	metal uptake	Slaveykova *et al.* (2003); Lamelas & Slaveykova (2007)
2. *Chlorella kesslerii* (freshwater alga)	Pb	metal uptake	Worms et al. (2010)
3. *Isochrysis galbana* (marine alga, no cell wall)	Pb	metal uptake; algal growth	Sánchez-Marín & Bieras (2011)
4. *Simocephalus serrulatus* (freshwater cladoceran)	Cu	mortality; metal uptake	Giesy *et al.* (1983)
5. *Daphnia magna* (freshwater cladoceran)	Cu	immobilization	Borgmann & Charlton (1984)
6. *Dreissena polymorpha* (zebra mussel)	Cd	metal uptake (whole body)	Voets *et al.* (2004)
7. *Pimephales promelas* (larval fathead minnow)	Cu	mortality	Erickson *et al.* (1996)
8. *Mytilus edulis* (marine bivalve)	Cu; Pb	metal uptake (whole body)	Lorenzo *et al.* (2005) Sánchez-Marín et al. (2011)
9. *Mytilus edulis* (excised gill) (marine bivalve)	Pb	metal uptake	Sánchez-Marín *et al.* (2007)
10. *Paracentrotus lividus* (sea urchin)	Pb	toxicity (embryo-larval growth)	Sánchez-Marín *et al.* (2007); Sánchez-Marín & Bieras (2012)

C – non-BLM behaviour, metal less available than expected

1. *Chlorella pyrenoidosa* (freshwater alga)	Al	growth inhibition	Parent *et al.* (1996)
2. *Paratya australiensis* (freshwater shrimp)	Cu	mortality	Daly *et al.* (1990)
3. *Salmo salar* (juvenile Atlantic salmon)	Al	mortality	Roy & Campbell (1997)

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10.2 Appendix B (Compilation of applications of the equilibrium adsorption (surface complexation) model to oxic sediments)

Table B- 1: 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 (drawn from MEND (1993); MEND (2009)).

Metal	Organism	Response	Geochemical predictor	Site	Reference
Cu, Pb	rooted aquatic plant, e.g. *Potamogeton richardsonii*	metal bio-accumulation	<Fe-OM>/<Fe-ox>	lakes, Rouyn-Noranda, QC	Campbell and Tessier (1991)
Zn	rooted aquatic plant *Vallisneria americana*	metal bio-accumulation roots	<Fe-OZn>/<Fe-ox> in the root plaque	fluvial lakes in St-Lawrence River, QC	St-Cyr and Campbell (1996)
Cd, Pb, Zn	rooted aquatic plant *Vallisneria americana*	metal bio-accumulation roots and foliage	$[Cd^{2+}]$ $[Pb^{2+}]$ $[Zn^{2+}]$ as estimated from oxic sediment-water equilibria	shallow fluvial lakes in St-Lawrence River, QC	St-Cyr and Campbell (2000)
Cu	filter-feeding bivalve *Elliptio complanata*	metal bio-accumulation	<Fe-OCu>/<Fe-ox> both extracted with $NH_2OH{\cdot}HCl$	lakes, Rouyn-Noranda, QC	Tessier et al. (1984)
Cd	filter-feeding bivalve *Pyganodon grandis*	metal bio-accumulation	$[Cd^{2+}]$, as estimated from oxic sediment-water equilibria	lakes, Rouyn-Noranda, QC	Tessier et al. (1993) Perceval et al. (2002)
Cd	filter-feeding bivalve *Pyganodon grandis*	metallothionein induction	$[Cd^{2+}]$, as estimated from oxic sediment-water equilibria	lakes, Rouyn-Noranda, QC	Couillard et al. (1993); Wang et al. (1999); Perceval et al. (2002); Giguère et al. (2003)
Cd, Zn	gastropod *Bithynia tentaculata*	metal bio-accumulation	$[Cd^{2+}]$ $[Zn^{2+}]$ as estimated from oxic sediment-water equilibria	shallow fluvial lakes in St-Lawrence River, QC	Flessas et al. (2000)

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10.3 Appendix C (Examples of Species Sensitivity Distribution (SSD) plots for the toxicity of individual metals)

Ag CCME 2015

Acute

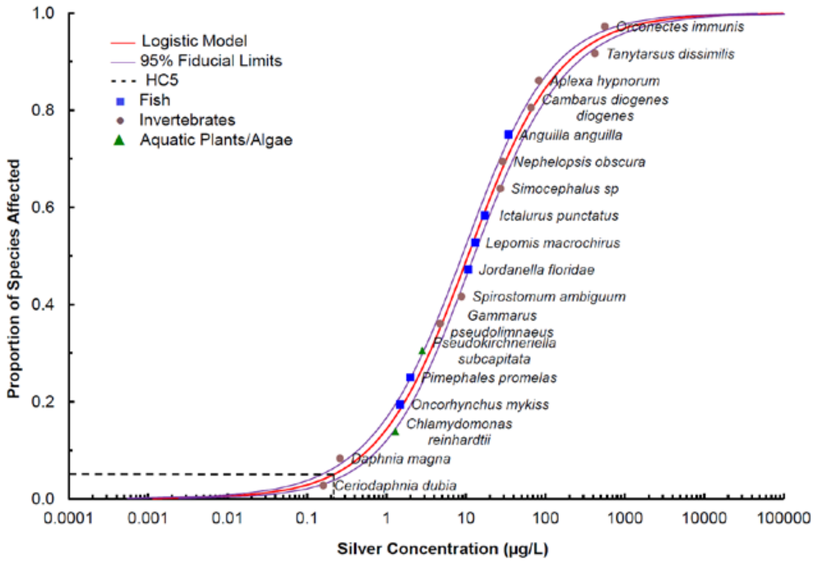


Figure 1. Short-term species sensitivity distribution (SSD) for silver in fresh water derived by fitting the log-logistic model to the short-term LC/EC$_{50}$s of 18 aquatic species.

Chronic

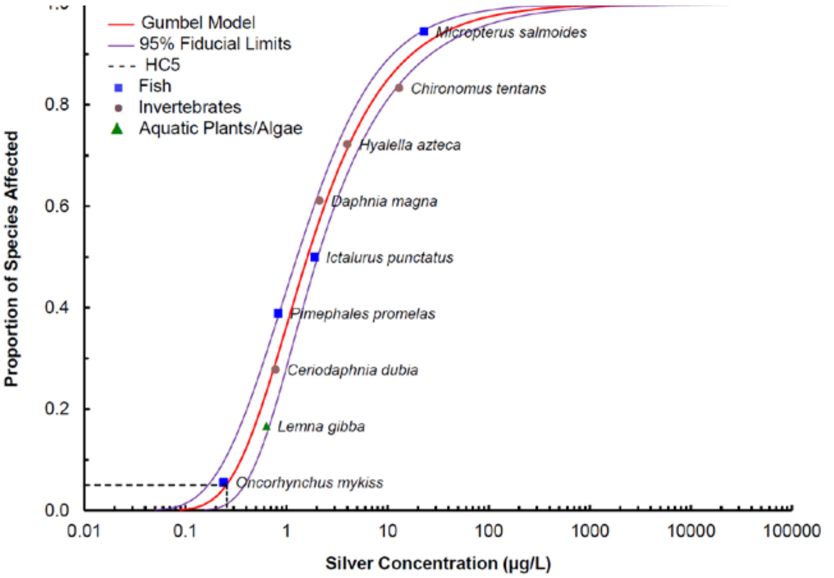


Figure 2. Long-term species sensitivity distribution (SSD) for silver in fresh water derived by fitting the Gumbel model to the long-term endpoints of 9 aquatic species.

Cd (Mebane 2010)

Acute and chronic

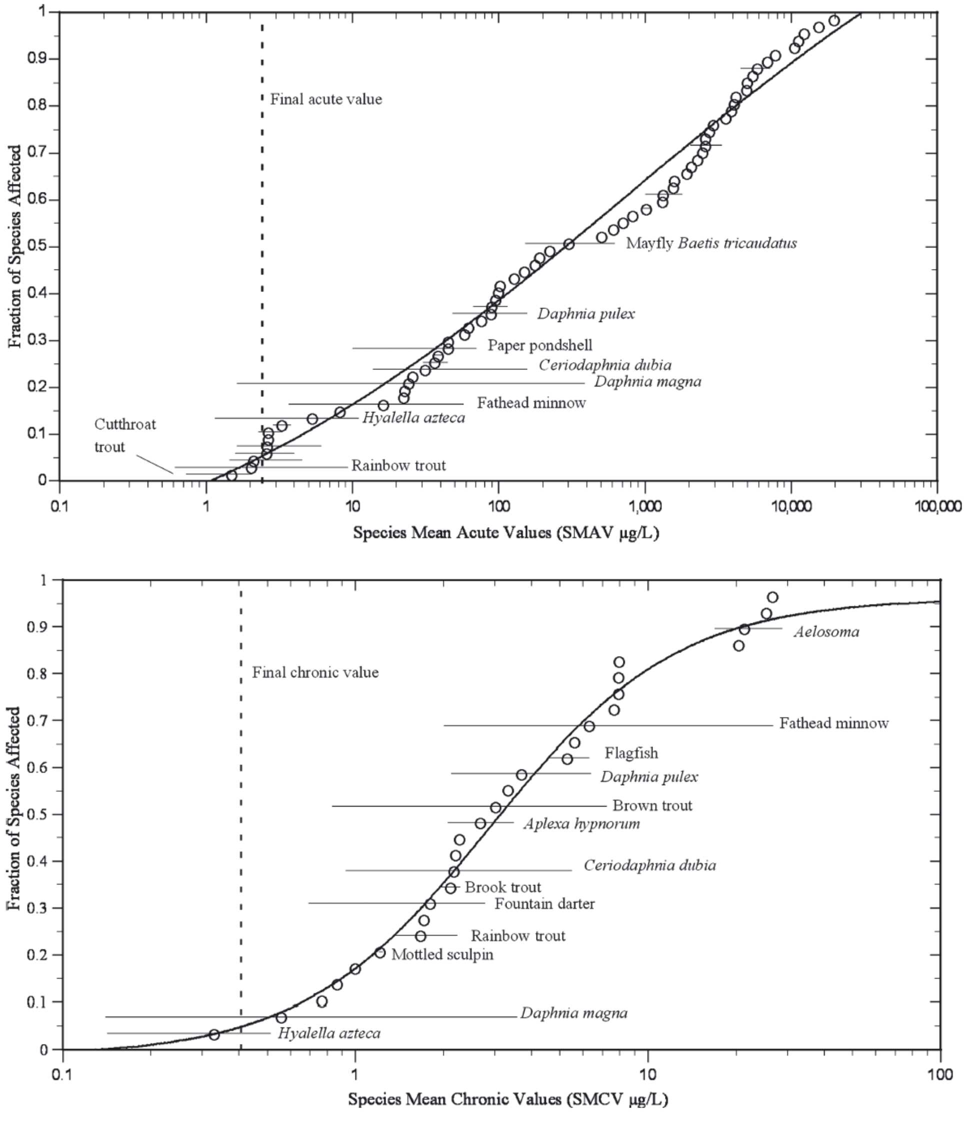
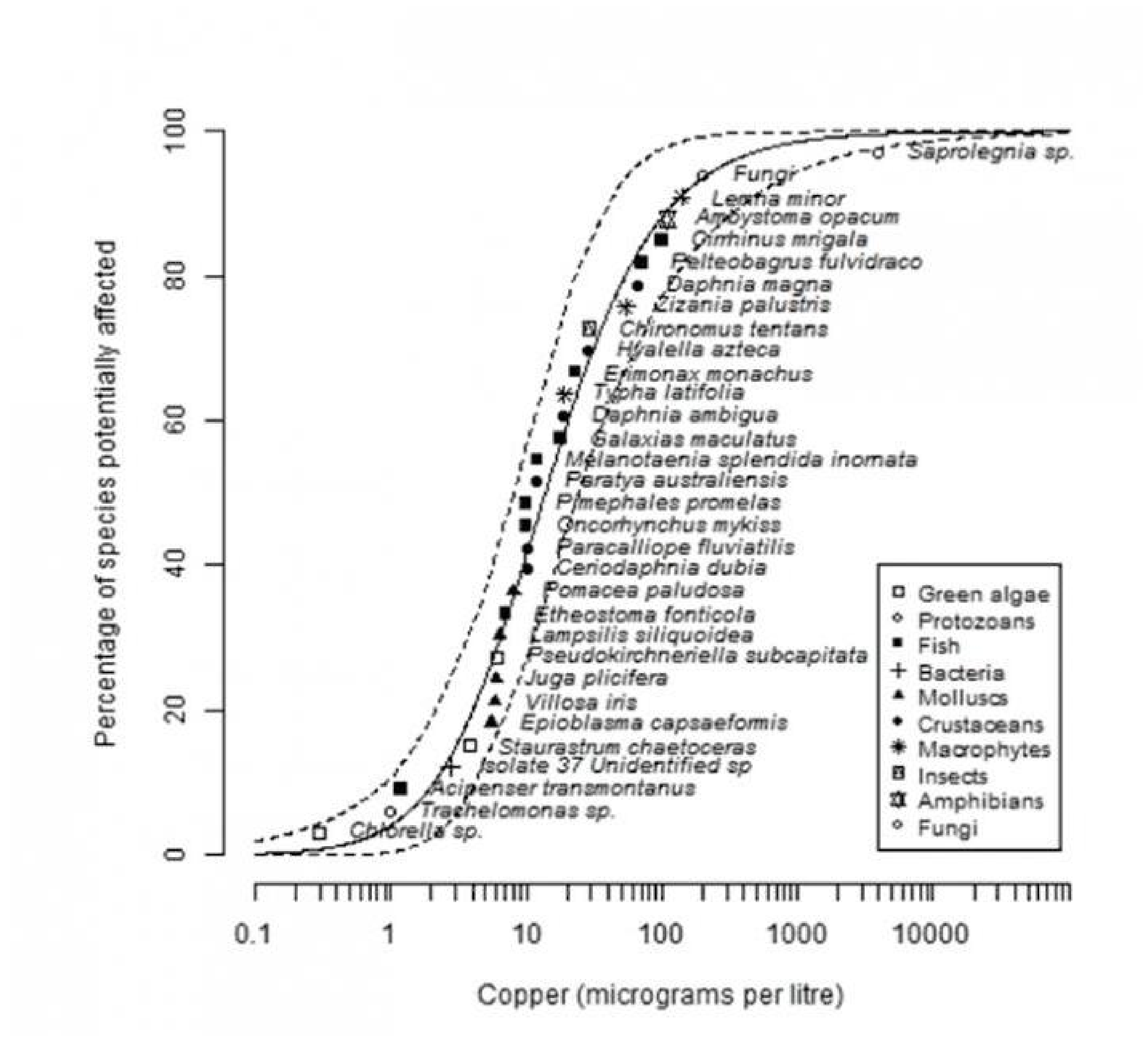


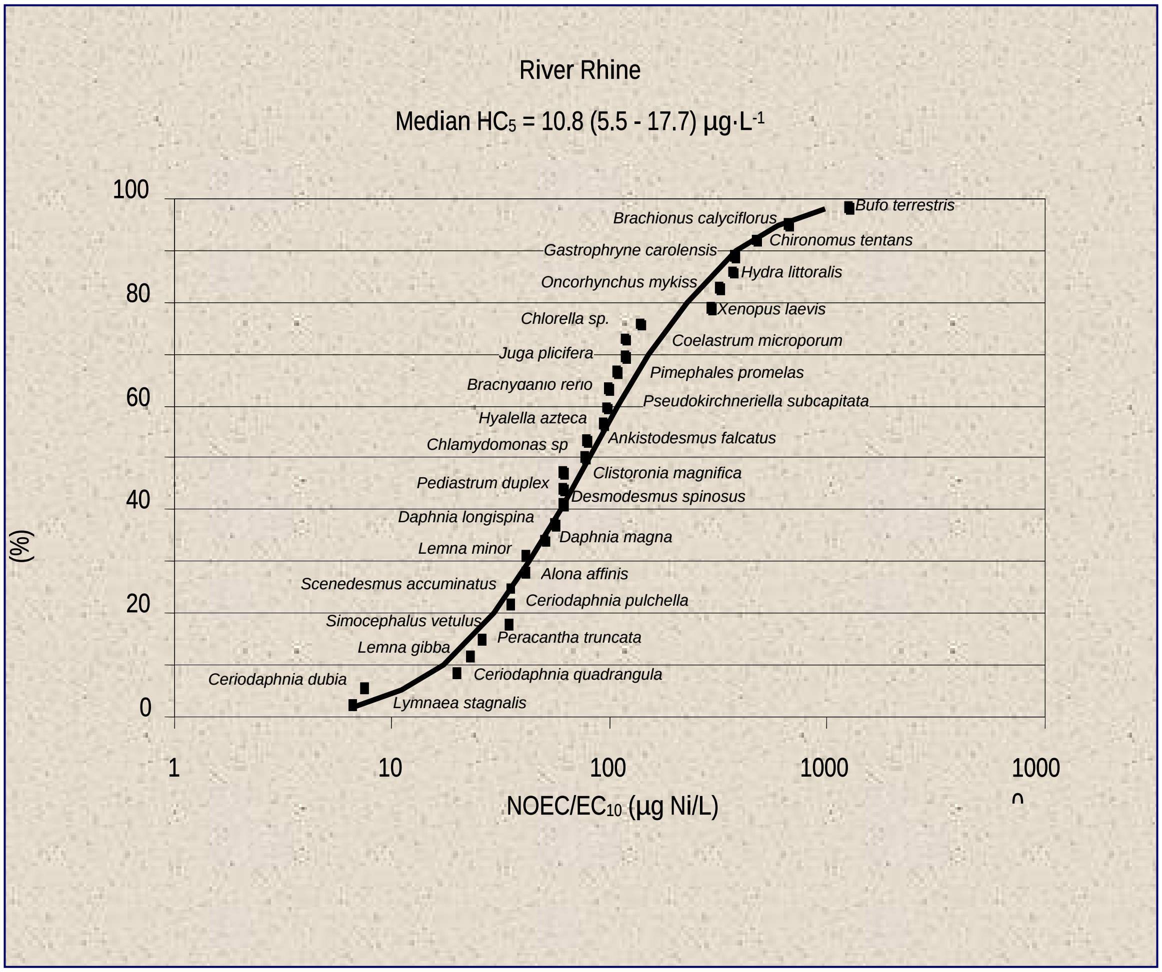
Fig. 1. Species sensitivity distributions of cadmium values showing species mean values (open circles), ranges of species values (horizontal lines), fifth percentile final acute and chronic values (vertical dashed lines), and their fitted logistic distribution curves. All values adjusted to a hardness of 50 mg/L $CaCO_3$.

Cu (ANZECC and ARMCANZ 2016a)

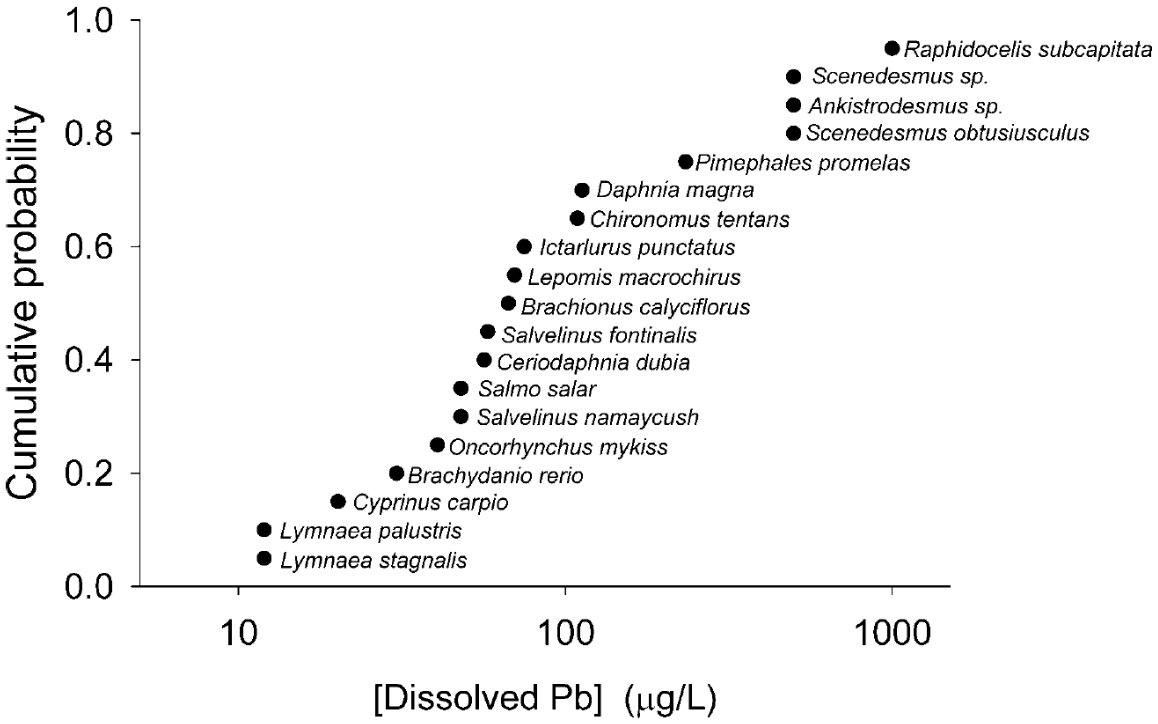
Chronic



Ni (NiPERA 2015)

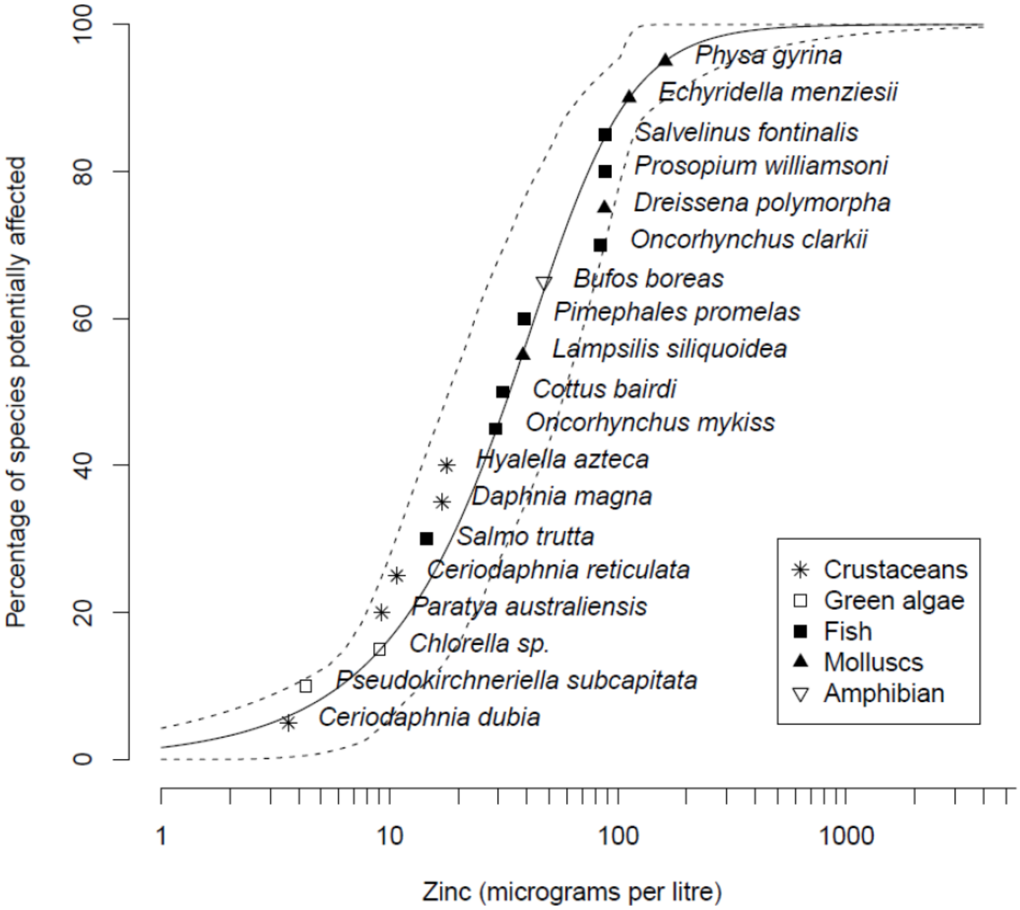


Pb (Grosell et al. 2006)



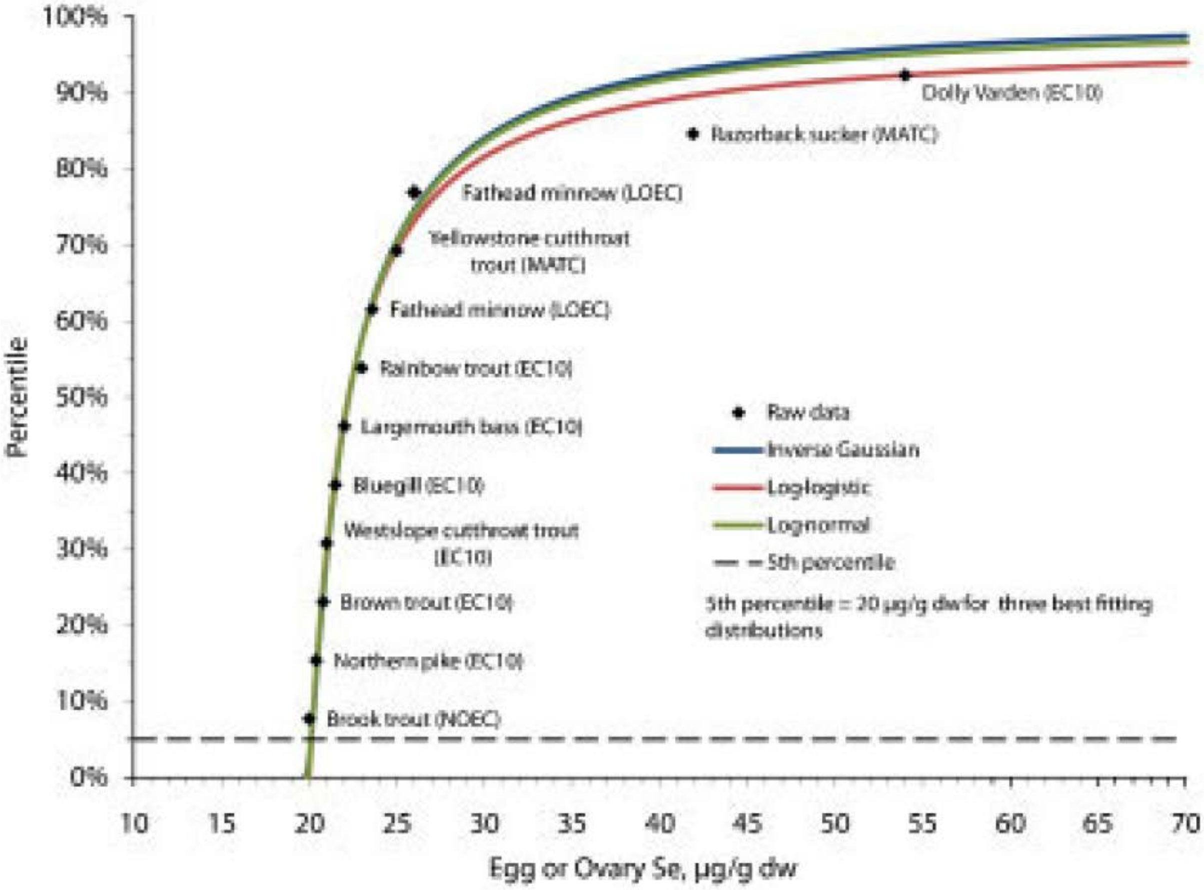
Zn (ANZECC and ARMCANZ 2016b)

Chronic



Se (De Forest et al. 2012)

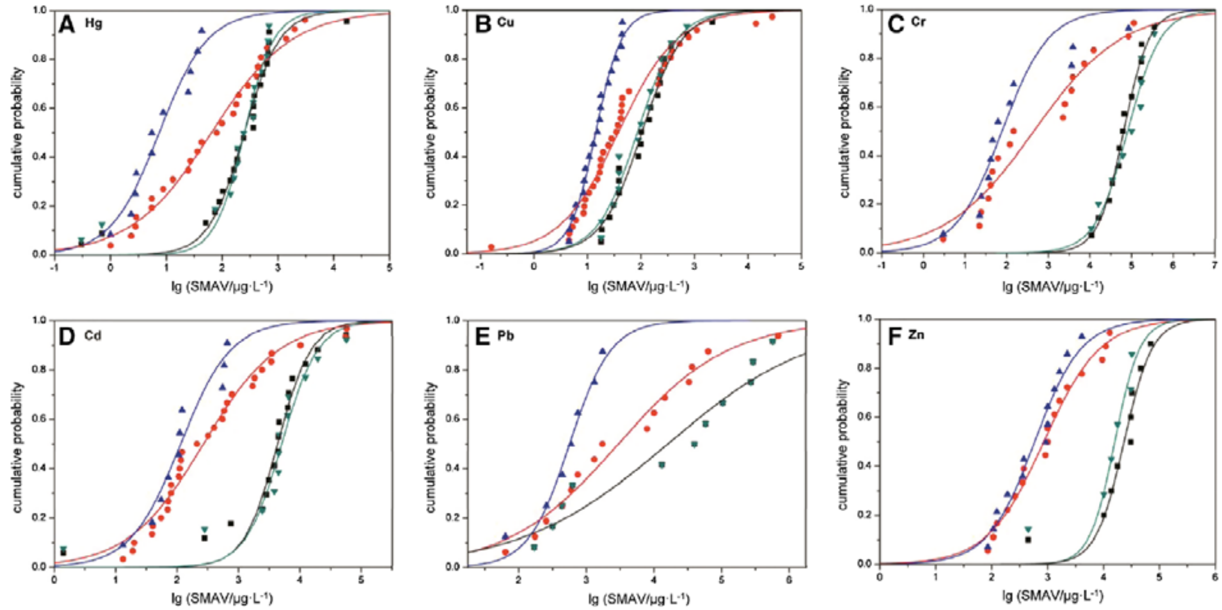
Chronic



Cd, Cu Cr, Hg, Pb, Zn (Zheng et al. 2015)

Comparison of species mean acute values (SMAV) for six metals, showing plots for different classes of aquatic animals:

triangles = cladocerans; inverted triangles = fish; circle = vertebrates; rectangle = invertebrates



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10.4 Appendix D (Reports, conference proceedings and slide presentations provided by NRCan-CanmetMINING and BC-MEND ML/ARD)

- Blier A. and G. Amyot 2011. Flooding as a reclamation solution to an acidic tailings pond and open pit; more than 15 years after. 18th Annual BC MEND ML/ARD Workshop, Vancouver, BC http://bc-mlard.ca/
- Davé N. 2008 Basic Understanding of Water Cover Applications and Case Studies, MEND Manitoba Workshop, Challenges in Acidic Drainage for Operating, Closed or Abandoned Mines, Winnipeg, June 4 and 5 http://bc-mlard.ca/
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10.5 Appendix E : Practical advice regarding geochemical and biological measurements recommended for SAD facilities and reference areas (Section 8).

Abiotic measurements (Sections 4.1-4.4)	Advice	Analytes	References
overlying water	• In-situ dialysis better than grab samples; can be combined with equilibrium ion exchange. Use data for BLM calculations. • Water samples also available from pore-water peeper cells that are above the tailings-water interface.[34]	Ca Mg Na K; Cl, SO_4, CO_3; pH, Fe, Mn, Al; DOC plus metals[33] of interest	Fortin et al. (2010); Crémazy et al. (2015)
	• Include field blanks; apply trace-metal-clean techniques.		Nriagu et al. (1993); Horowitz et al. (1994)
	• Include measurements of algal productivity (direct chlorophyll-a measurements or *in situ* fluorimeter profiling) to establish trophic status.	plant nutrients (NH_4^+, NO_3^-, PO_4^{3-}) chl-a	AETE (1997b)
	• If the presence of process chemicals is suspected, include them and their degradation products.	thiosalts; process chemicals and their degradation products	
pore water (see Table 4-1)	• Pore-water peepers the preferred approach (they yield depth-profiles of pore-water concentrations, within the deposited tailings and also across sediment-water interface).	Ca Mg Na K; Cl, SO_4, CO_3; pH, Fe, Mn, Al; DOC plus metals of interest; O_2; $\Sigma S(-II)$; $\Sigma S(0)$; thiosalts; process chemicals and their degradation products.	Carignan et al. (1985); Carignan et al. (1994); Martin et al. (2003a); (2003b; 2003c); Wang and Tessier (2009)
	• DGT samplers also used, but link of DGT-labile metal to bioavailability in sediments is tenuous (see Section 2.2.6).	Cu Ni Pb	Martin and Goldblatt (2007); Martin (2008); Stahl et al. (2012); Yin et al. (2014)

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[33] For simplification in this table, the term 'metal(s)' includes 'oxyanion(s)'.

[34] Similarly, the term 'tailings-water interface' includes 'sediment-water interface'.

		As Sb; Fe	Bennett et al. (2012); Arsic et al. (2018)

	Advice	Analytes	References
sediments/ tailings (see Table 4-1)	• Teflon strips used to collect authigenic Fe and Mn oxyhydroxides and their associated metals.	Fe Mn; As Mo Sb	Belzile et al. (1989); Fortin et al. (1993); Feyte et al. (2010)
	• Use penetrometer to determine suitability of sediments for burrowing invertebrates.		Bokuniewicz et al. (1975); Crane and Merz (2017)
	• Short cores, taken across the tailings-water interface; preserved cold or frozen, not dried. • Use partial extractions (see Section 4.3).	organic carbon; SEM, AVS; amorphous iron oxyhydroxides + associated metals, including Fe, Mn	Tessier (1992); Tessier et al. (1993); Garforth et al. (2016); Ren et al. (2016)
Biotic measurements (Section 4.5, Table 4-4)			
periphyton	• Collect using artificial substrates (e.g., honed stone tile – H. Larratt, personal communication).	metals of interest + Al Fe	AETE (1997b); DeNicola and Stapleton (2002); Haack and Warren (2003); Vigneault et al. (2007); Lopez et al. (2016)
	• Periphyton communities are sensitive to environmental conditions; a mining effect may be masked by variations in light, nutrient or grazing regimes. • Include determination of ash-free dry weight and analysis of Al and Fe as indicators of ‘contamination’ by inorganic particles.		
rooted aquatic plants	• Collect when plants are mature, before seasonal decline.	metals of interest + Al Fe, in foliage and roots	
	• For roots, measure with and without treatment of		St-Cyr and Campbell (1996); AETE (1997b);

	root surface to remove iron oxyhydroxides. • Depending on plant species, metals may be taken up via the roots, via the foliage, or both (see 4.5.1).		St-Cyr and Campbell (2000)
benthic invertebrates	• Collect before insect emergence and at the same season every year.		
	• Include members of benthic community with different feeding/living strategies – these will affect their exposure to metals (e.g., epibenthic and endobenthic macroinvertebrates; grazers, deposit feeders, carnivores).	metals of interest + metal-specific biomarkers of exposure and effect	Warren et al. (1998), Hare et al. (2001) Proulx and Hare (2009)
	• Include filter-feeders (bivalves) or grazers (gammarids) as a measure of the bioavailability of metals in the water column.		AETE (1997a); Perceval et al. (2006); Uher et al. (2018)
	• Other criteria to consider: ▪ relatively sedentary invertebrates (their metal content will reflect the bioavailability of these elements in the local environment); ▪ invertebrates large enough to allow measurements of trace metals in individual organs; ▪ invertebrates representing range of metal concentrations to which fish might be exposed in their diet.		
	• Allow bottom-feeding benthic invertebrates to clean out their guts by keeping them alive in		

ambient water for 24 -48 h.

	• Benthic invertebrates can also be used to develop community indices of abundance, diversity, richness (but sampling strategies are different)		AETE (1999b); AETE (1999a)
fish	• Emphasis on bottom-feeding fish, i.e., those most closely linked to the benthic community – see Table 4-4.	metals of interest + metal-specific biomarkers of exposure and effect	Harrison and Klaverkamp (1990); Miller et al. (1992); AETE (1997a); AETE (1999c); Giguère et al. (2004); Kraemer et al. (2008); Janz et al. (2014); Couture et al. (2015)
	• Consider using small-bodied fish with small home ranges (e.g., sculpins) that remain in the target area and do not migrate. • Other criteria to consider: o age o season (spawning cycle) o specific organ (gill, liver, gonad,…) • Avoid stainless steel implements for dissection (use high carbon implements to avoid contamination).		

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