Biochemical and morphometric effects of industrial metal contamination on wild yellow perch

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The global objective of this project was to understand the extent of metabolic impairment and factors that influence tissue metal accumulation, growth, longevity and physiological condition in wild yellow perch (YP) inhabiting metal-contaminated lakes from two geologically similar, but geographically distinct, metal gradients (Sudbury ON and Rouyn-Noranda QC). Sampling occurred in five lakes near Sudbury (2003), and another five near Rouyn-Noranda (2004), in June and August. Using a range of sampling gear, at least 120 YP were sampled from each lake during each sampling event, representing the full size/age range of each population. Age, length, and mass were recorded for each fish, and condition factor was determined. Each fish was digitally photographed and ecomorphometric condition was assessed using truss networks analysis. Metal concentrations were measured in stomach contents, liver, and kidney of a subset of YP from each age class. Liver and white muscle aerobic (using citrate synthase and cytochrome C oxidase activity) and anaerobic capacities (using lactate dehydrogenase) were also measured for each subsample. Water and sediment contamination of each lake was determined. In 2003, an additional 55 fish were sampled live from each of three variously-contaminated lakes, and were maintained in dechlorinated laboratory water to examine aerobic and anaerobic recovery, tissue metal depuration, and indicators of protein synthesis (liver and muscle protein contents and nucleoside diphosphate kinase activity, muscle RNA/DNA ratio).

This study yielded the largest database of its kind, including over 2000 YP from 10 lakes in 2 gradients and 2 seasons, and over 57,000 data points. Our data suggest that there are important regional and seasonal variations in dietary and tissue metal concentrations, and morphological and metabolic condition. Metal contamination in YP tissues largely reflected their environment. Although, diet had a major influence on tissue metal concentrations, our analysis failed to reveal evidence that ontogenetic diet shifts affect dietary metal uptake. On the contrary, dietary metal concentrations in YP from contaminated lakes appeared highly variable but consistently elevated relative to food of clean fish. Except for selenium, the laboratory depuration study revealed that tissue metal concentrations do not decrease when fish are provided with cleaner conditions. Relationships between tissue metal contamination and fish condition were unclear. Although we confirmed that elevated Cu contamination is associated with decreased muscle aerobic capacities, our results suggest that Ni contamination may lead to an increase in aerobic capacities and a decrease in condition and longevity. Both field and lab studies support that morphometric and metabolic condition are strongly influenced by biotic (fish size) and abiotic (seasonal factors) variables, respond differently in different regions, and are also affected by metal contamination.

This study will provide understanding of the myriad factors influencing metal accumulation and subsequent effects on YP from contaminated environments. Consequently, this research will contribute to the development of new biomarkers for metals ERA that are relevant in the field for indigenous fish species of Canadian mining areas.

MITE-RN was the major funding source for this project.

Project C.1: Predicting metal and metal mixture effects in *Hyalella azteca*. Bioaccumulation and toxicity in Metal Mixture Modeling, Biotic Ligand Modeling, and Site Assessments.

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Total metal concentrations in the environment do not provide accurate estimates of toxicological effects since toxicity is a function of metal speciation and bioavailability. The main objectives of this project are to determine 1) the best methodology for accurately predicting single metal effects and 2) the most appropriate method of quantifying the effects of metal mixtures. Four studies, all using the benthic amphipod *Hyalella azteca*, and all comparing toxic effects to bioaccumulation, address these objectives:

A. Bioccumulation (1-week tests) and chronic toxicity (4-week tests) of mixtures of 7 to 10 metals are being determined. The interactive effects on metal accumulation are being compared with those on toxicity to determine if toxic effects can be predicted more reliably from body concentrations rather than water concentrations. To date, toxicity-bioaccumulation relationships for single metals have been determined for As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl, and Zn. Several experiments with mixtures have been completed, including measurement of the effects of each metal on the bioaccumulation of the other metals, and chronic toxicity of the 10-metal mixture.

B. A Biotic Ligand Model (BLM) has been produced to explain major ion effects on Ni bioaccumulation and toxicity. The fundamental tenet of the BLM (equal bioaccumulation results in equal toxicity) appears to be valid for 1-week test. Additional experiments appear to validate the BLM for chronic toxicity. If metal interaction effects are observed in study A, the BLM can be expanded to produce a metal-mixture BLM. Progress has also been made towards the development of a BLM for Cd in *Hyalella*.

C. The study on the effects of overlying water on metal toxicity in sediments from Sudbury and Rouyn-Noranda area lakes has been completed, and a M.Sc. thesis has been completed. Overlying water has a strong effect on Cd bioavailability, but not on Ni bioavailability because major ion effects on Ni accumulation cancel major ion effects on water-sediment partitioning. The data, based on field-collected water and sediments, appear to validate the metal-mixture BLM produced under studies A and B.

D. A new study was initiated on bioaccumulation and effects from dietary Cd accumulation in year 3 of MITE-RN. Algae have been cultured in the presence of Cd to produce a “naturally” contaminated diet. Chronic toxicity studies are currently underway with *Hyalella*. The objective is to determine if Cd accumulated from food is equally toxic as Cd accumulated from water. This has important implications for interpreting the significance of Cd bioaccumulation and performing risk assessments for Cd.

Direct (physiological) and indirect (food-web mediated) effects of metal exposure on yellow perch

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Attempts to define the impacts of metals on aquatic ecosystems have usually involved laboratory experiments under defined conditions (toxicity tests) and, to a lesser extent, field observations on impacted indigenous populations. To link these two approaches, one needs a common measure of metal exposure in laboratory and field settings. The determination of metal concentrations or burdens in tissues (or whole organisms) has been suggested as a means of achieving this linkage. In this project we have explored this approach with indigenous fish, having expanded the concept of metal "body burden" to take into account the speciation of the metal within the organism, i.e., the organism's ability to detoxify the metal. The project was designed to look for metal-induced effects on a biosentinel species, yellow perch (YP: *Perca flavescens*), in lakes located along an existing metal gradient, downwind and downstream from past/current metal smelters. We looked for evidence of direct physiological effects, and for food-web mediated bioenergetic effects. In the case of **direct** effects, a key hypothesis to be tested was that there exists a mechanistic link between the intracellular speciation of the metals and the manifestation of deleterious effects at the organism (physiology, endocrine and metabolic status, growth, reproductive status) and population (abundance, production, reproductive fitness) levels. Specifically, we investigated the following linkages: chronic metal exposure → metallothionein induction → perturbed intracellular metal partitioning → endocrine / physiological impairment → diminished growth efficiency → reduced survival, altered population age structure and population dynamics. In the case of **food-web mediated** effects, we focused on the following sequence: chronic metal exposure → reduced food abundance of certain dietary components → increased energetic costs of feeding → reduced growth efficiency and ultimately stunting, i.e., we looked for evidence of "energetic bottlenecks" imposed by the absence of key prey components that are necessary for normal diet shifts and growth to occur. Different life stages of yellow perch (*Perca flavescens*) were chosen as trial biosentinel organisms (young-of-the year, juvenile 1+ fish, adults). Much of our field work was devoted to sampling these different life stages from lakes located along the metal exposure gradient, and characterizing them biochemically and physiologically. However, we also performed reciprocal transfers of juvenile fish between low-metal and high-metal exposure lakes, and carried out experimental feeding trials with natural food from low-metal and high-metal lakes.

In lakes at the high end of our exposure gradient, metals (Cu, Ni and especially Cd) accumulated in YP to concentrations well above background tissue values; increases in tissue Zn concentrations were much more modest, despite the existence of a very marked gradient in ambient [Zn]. YP clearly handle essential and non-essential metals differently. Feeding experiments suggested that both water and food contribute to Cd accumulation in juvenile YP.

Metal accumulation in YP is accompanied by metallothionein induction, but detoxification of Cd, Cu and Ni by metallothionein is incomplete. Indeed, direct effects of metal toxicity were detected at multiple levels of biological organization, from effects at the cellular level, to effects in organs and tissues, to individuals and populations, in a pattern linked to accumulated metal concentrations (i.e., along the exposure gradient). In the Rouyn-Noranda area, metal-induced effects appear to be most closely related to Cd bioaccumulation; this is not the case in the Sudbury area, where Ni (and Cu?) are the metals most closely related to the sub-lethal effects observed in YP.

In addition to direct or physiological effects, we also documented indirect, food-web mediated effects of metals on YP in the most contaminated lakes. The most common indication of such indirect effects on YP is severely stunted growth coupled with a high degree of zooplankton dependence throughout their life.

The absence of a clear threshold metal concentration, below which yellow perch are able to detoxify incoming metals (i.e., the apparent failure of metal "spillover theory" to apply under chronic exposure conditions), runs counter to prevailing thinking about metal toxicity. The demonstration of food-web mediated effects of metals on consumers serves as a reminder of the importance of the "less-noble" or forage aquatic species, i.e. those that serve as the prey base for the top consumers.

Extending the Biotic Ligand Model (BLM) approach for assessing chronic impacts of metals in freshwater fish: prospects and challenges

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The Biotic ligand model (BLM) is an elegant and cost-effective framework for deriving site-specific water quality guidelines for cationic metals. The BLM approach is based on an understanding of the key toxic mechanisms of action of metals at the gills, and relates the predicted gill burden in a given water chemistry to predicted toxic effect. Present versions of the BLMs are designed to protect against acute waterborne toxicity of metals. However, in many jurisdictions, including those of Canada, the regulatory focus is gradually shifting to investigate the potential of the BLM approach for assessing chronic impacts of metals in aquatic environment, and thereby providing lifetime protection to resident fauna. We believe that future chronic BLMs should not be developed through simple recalibrations of the present acute BLMs (e.g. by use of acute-to-chronic ratios, as currently proposed by U.S. EPA) but rather should be developed from experimental chronic toxicity data. This is primarily because the mechanisms of acute and chronic toxicity of metals are not always the same, and both chronic waterborne and dietary factors should be taken into account for assessing chronic metal toxicity in addition to water chemistry. The goal of our MITE-RN funded research was to assess the importance of different real world factors such chronic pre-exposure to waterborne and dietary metals, different water chemistry conditions (hardness) and dietary quantity and quality (essential ion content) in influencing tissue-specific metal accumulation and chronic toxicity of metals in freshwater fish. The metals we investigated are Cu, Cd and Zn, and we used a model salmonid, rainbow trout (*Oncorhynchus mykiss*), for laboratory-based controlled exposure studies, and an endemic percid of the metal-impacted waters of eastern Canada, yellow perch (*Perca flavescens*), for lab-to field validation. This presentation will provide an overview of some of the most important findings of our MITE-RN project, illustrating that (i) ionic homeostasis under both chronic waterborne and dietary metal exposures is maintained primarily through the modulation of gill physiology; (ii) gill-metal binding characteristics (affinity and capacity) can profoundly change under the influence of natural factors such as chronic pre-exposure to waterborne and dietary metals, dietary ion contents, and different water hardness, thereby suggesting that properties of the biotic ligand are not fixed but rather dynamic, contrary to the basic assumption of the present BLM approach; and (iii) tissue-specific metal accumulation and toxicity can be greatly influenced by dietary quantity as well as quality. Overall, these findings suggest that the present BLM approach can be extended for assessing chronic toxicity of metals in freshwater fish, as long as these factors are taken into account. (funded by MITE-RN).

MITE-RN Domain: Impacts Platform Presentation

How does research on the invertebrate, *Hyalella azteca*, fit into ecological risk assessment?

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It is essential that accurate and reliable methods are developed that identify environmental concentrations of metals above which adverse biological effects occur. In the past, environmental quality criteria have been based primarily on total metal concentrations in water or sediment. These measures do not provide accurate estimates of effects since toxicity is a function of metal bioavailability. Bioavailability is a function of both chemical and biological processes; therefore this research has focused on these aspects using the epi-benthic, invertebrate *Hyalella azteca*.

A number of methods have been developed which are important to Ecological Risk Assessment (ERA). First, a Biotic Ligand Model (BLM) for Ni toxicity to *Hyalella* has been developed. This model assumes that toxicity is a function of metal bioaccumulation, which is a function of cations in the water competing with Ni for binding sites on the biotic ligand. Secondly, the relationships between bioaccumulation and toxicity of 10 metals have been modeled individually and Critical Body Concentrations (CBC's) determined. From these relationships, a Metal Effects Addition Model (MEAM) for predicting the impacts of metal mixtures has been developed and tested. As well, changes in metal bioaccumulation due to interaction between metals in mixtures have been investigated. Finally, bioaccumulation and toxicity tests to assess site water and sediment have been employed, from which Toxicity Identification Evaluation (TIE) was possible utilizing CBC's. A prediction of risk utilizing the BLM and the MEAM was done. Results were also compared with benthic community assessments.

The methods developed in this project can provide site specific information, identify adverse effects, identify metals of concern, predict effects of bioaccumulated metals, and link laboratory based models to effects observed in the field.

A field study examining metal depuration kinetics in juvenile yellow perch (*Perca flavescens*)

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The objective of our study was to measure trace metal efflux from juvenile yellow perch (*Perca flavescens*) in nature. To achieve this goal, we set up 12 mesh cages (volume = 5.6 m^3) in each of two lakes located in the Rouyn-Noranda region of northwestern Quebec; contaminated Lake Dufault and uncontaminated Lake Opasatica. The 0.5 cm mesh size of the cages permitted zooplankton, the major food of juvenile perch, to freely move in and out of the cages. Juvenile perch caught in Lake Dufault were either transplanted to Lake Opasatica or caged within their native lake as a control. Approximately 50 fish (1.03 ± 0.06 g wet weight) were added to each cage and they were then sampled after 0, 10, 20, 40, 50, 60 and 75 days. The liver, kidney, gills, and gut were removed and analyzed for metals. In fish transferred to the uncontaminated lake, Cd concentrations decreased gradually in the gills, gut and liver to reach values that were 93%, 82% and 66% lower, respectively, relative to controls. In contrast, little Cd was lost from the kidneys during our 75-day experiment. Similarly, Cu concentrations declined by 30% in the liver of transplanted fish after 75 days, whereas Cd concentrations in the kidneys remained stable. Cu concentrations in the gills and gut were slightly lower in transplanted fish than in controls. Zinc concentrations were also marginally lower in the gills and the gut of transplanted fish, but similar in the liver and kidneys of transplanted and control fish. Our study suggests that (i) once placed into a reference environment, concentrations of metals in the gills and gut decrease, illustrating either a loss of metals from these organs to the external environment, or a translocation of these metals into other organs; (ii) under natural conditions, there is little loss of metals from the kidneys of perch; and (iii) loss of Cu and Cd from the liver suggests the importance of biliary excretion as a route of elimination for these metals.

A field study examining the relative importance of food and water as sources of Cd for juvenile yellow perch

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The objective of this field study was to determine the relative importance of water and food as Cd sources for juvenile yellow perch. The perch were caged within either a reference (Lake Opasatica) or a Cd-contaminated lake (Lake Dufault). The cages were designed to allow free exchange of water between the cages and the lake, while restricting the movement of zooplankton. The fish were fed with zooplankton from either the reference or Cd-contaminated lake creating 4 treatment groups: reference; Cd-contaminated food only; Cd-contaminated water only; and Cd-contaminated food and water. The gills, gut, kidney and liver were sampled after 15 and 30 days of exposure and these organs were measured for Cd. In addition, uncaged indigenous perch from both lakes were sampled on day 0, 15 and 30. Results demonstrated that the gills, gut and kidney were all influenced largely by aqueous Cd, whereas the liver appeared to respond to both dietary and aqueous Cd. To further examine Cd accumulation at the subcellular level, differential centrifugation was carried out on the liver, yielding fractions containing cellular debris, granules, organelles, heat-denatured proteins and heat-stable proteins. In uncontaminated Lake Opasatica fish, the majority of the Cd burden was associated with organelles and heat-denatured proteins (metal-sensitive fractions), whereas in Lake Dufault fish the majority of the Cd was associated with heat-stable protein fractions (metal-detoxified fractions). The importance of the metal-detoxified fractions in fish exposed to contaminated food only, or contaminated water only, became increasing important as hepatic Cd concentrations increased, thus underlining the importance of these fractions in a Cd-contaminated environment. The importance of both food and water as a Cd source in this field study suggests that models designed to predict Cd concentrations in yellow perch will be more reliable if they consider both dietary and aqueous Cd.

Effect of Cu on stress-induced physiological responses in rainbow trout (*Oncorhynchus mykiss*).

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Fish are exposed to a number of stressors in their habitats. To evaluate the capacity of Cu, an essential metal, to act as a chemical stressor, rainbow trout (*Oncorhynchus mykiss*) were exposed to Cu (30 or 80 µg/l) for 30 days in the laboratory and they were also submitted to a confinement stress before sampling. Fish were submitted to control conditions (no Cu, no confinement), exposure to Cu alone, physical stressor alone (confinement), or both Cu and physical stressor (Cu/confinement). Fish exposed to Cu only had a lower CF, HIS, plasma glucose, hepatic glycogen and gill gill Na^+/K^+ ATPase compared to controls. Confinement increased plasma cortisol in all groups of fish but this effect was lower in fish exposed to Cu. There were no significant differences in plasma cortisol levels between fish exposed to Cu or not, sampled without confinement.

To better characterize effects of Cu on cortisol secretion, adrenocortical cells were isolated from the head kidney and exposed to Cu *in vitro*. The cells isolated from trout exposed *in vivo* to Cu and subjected to the additional stress of confinement, had the lowest capacity to secrete cortisol once exposed *in vitro* to Cu. Our results show that long term exposure to Cu alters several physiological biomarkers and impairs cortisol secretory capacity of the adrenocortical cells in fish.

Modelling Toxicity of Nickel to *Hyalella azteca* using a Biotic Ligand Model Approach

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As part of the Metals in the Environment Research Network, a model was developed to predict nickel toxicity to *Hyalella azteca*, a freshwater amphipod, in short term and long term exposures using the biotic ligand model approach. A model using an invertebrate in longer-term exposures provides a sensitive indication site-specific effects of metals and may be used in standards setting or in risk assessments. To develop the model, short-term (7-day) bioaccumulation experiments in various media were performed with adult *Hyalella* to determine which ions influenced nickel uptake. Calcium and hydrogen were found to be the only important competitors with nickel and were incorporated into a saturation equation where the tissue nickel concentration was estimated from the concentration of calcium, hydrogen and nickel, their respective binding constants as well as the total ligand available for binding. The model was validated by comparing toxicity predicted by the model to that observed in toxicity tests performed over both short- and long-term exposures using young *Hyalella*. Bioaccumulation was related to toxicity by comparing tissue concentrations, predicted by the model under given exposure conditions, with those corresponding to observed LC50s. The relatively good agreement between predicted and observed toxicity, as well as the stability of the tissue concentration predicted at various LC50s showed that the biotic ligand model approach is suitable for predicting nickel toxicity to *Hyalella*.

Metal mixture impact: Concentration (Toxic Units) addition vs effects addition. Interactive effects of mixtures on bioaccumulation and toxicity in *Hyalella azteca*.

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Mortality rates for chronic (4-week) toxicity were used to determine interactive effects on toxicity. A concentration series of "equi-toxic" mixtures of ten metals (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Tl & Zn) was tested. Bioaccumulation of each metal and total survival at each mixture treatment was determined. The observed toxicity was then evaluated through a comparison of the "Concentration Addition Model" using toxic units (CAM-TU) versus the "Metal Effects Addition Model" (MEAM), based on measured water and body metal concentrations. Observed survival was greater than expected based on the CAM-TU and less than expected based on the MEAM. Overall, the MEAM predicted survival more accurately, especially based on total body concentrations.

Interactive effects of metal mixtures on bioaccumulation were also determined from one-week exposures. Metal mixtures were produced with "equi-toxic" concentrations of each metal. Interaction effects were determined by comparison of each metal's bioaccumulation from individual exposures to that in exposures with a matrix of different mixtures based on seven metals (As, Cd, Co, Cr, Ni, Pb and Tl). Copper, Mn and Zn were not included in the first set of tests since they could confound the results due to partial regulation of Cu and Zn by *Hyalella* and also the high concentrations of Mn required at its "equi-toxic" concentration. Therefore a second matrix of metal mixtures were tested, based on the seven metal above, in combination with: Cu, Mn and Zn individually, the binary pairs, Cu-Mn, Cu-Zn and Mn-Zn, and finally the tertiary group Cu, Mn and Zn, thus generating the full, 10-metal mixture. Cd, Co and Tl bioaccumulations were significantly reduced in the seven metal mixture whereas Pb accumulation was significantly increased. Cd, Co and Ni accumulations were further and significantly reduced in the 10 metal mixture exposure. Therefore, significant interaction affects on metal accumulation do occur in mixture exposures.