MITHE-SN ANNUAL SYMPOSIUM

P.I. Platform Abstracts

Project Title:
(A1) Generation and field validation of chronic biotic ligand models for fish: a synthesis of MITHE

Investigator(s):
[1]Pyle, G.G., [1,2]Mirza, R.S., [2]de Francesco, M., [1,2]Green, W.W., [1]Ryan, P., [2]Wood, C.M.

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Summary:
In order for the Biotic Ligand Model (BLM) approach to be useful in an Ecological Risk Assessment (ERA) context, chronic BLMs are required to reflect the contamination realities of natural systems. Project A1 in the MITHE-SN has been working towards developing two non-traditional BLMs involving epithelia of the digestive and olfactory systems in both lab-reared (fathead minnows, *Pimephales promelas*) and wild fishes (yellow perch, *Perca flavescens*). Focus on gut epithelium reflects that a significant source of metal uptake to fish occurs through the diet. Focus on the olfactory epithelium reflects the ecological significance of chemosensory-mediated behaviours in natural systems, and that metals are powerful chemosensory inhibitors.

Wild yellow perch collected from metal-contaminated freshwater systems near Sudbury, ON, demonstrate that BLM parameters for Zn-binding capacity (B_{max}) and affinity (*log K*) to gill epithelium varies in fish collected from clean and contaminated lakes. However, no differences B_{max} or log K were detected in gut epithelium, suggesting that the gill is more important for Zn uptake than gut. In a series of experiments involving an *in vitro* gut-sac assay using wild yellow perch from a clean lake to examine the influences of dietary Ca on dietary Cu uptake, we demonstrated that the fluid transport rate and Cu absorption through the gut decreased with increasing dietary Ca concentrations, although results were not statistically significant owing to high variability among experimental treatments. Experimental results examining dietary Cu uptake kinetics demonstrated that wild yellow perch demonstrated higher maximum Cu uptake rates (J_{max}) and lower Cu-binding affinity (i.e., higher K_m) than previous reports for rainbow trout (*Oncorhynchus mykiss*). Finally, unlike rainbow trout, elevated Na concentrations in yellow perch diet did not affect Cu uptake dynamics. However, increased dietary Fe concentrations caused an increase in the amount of Cu taken up to the intestinal epithelium, but a reduction in the amount of Cu released to the serosal solution (representing transport across the epithelium) in the gut-bag assay. This result suggests that dietary Fe may have an influence on Cu uptake, which could be relevant in natural environments contaminated by mining effluents high in Fe.

Our developmental work on a chemosensory-based BLM will be presented in a poster by Mirza et al. Briefly, wild fathead minnows from a contaminated lake failed to learn predator (smallmouth bass) cues whereas those from a clean lake did. This resulted in decreased survival of contaminated fathead minnows in staged predator-prey trials relative to those from a clean lake. Using a reciprocal cross-exposure experimental design in clean and contaminated lake water, minnows from the contaminated lake could not respond to chemical alarm cue, even after being held in clean water for up to 37 d, suggesting long-term chemosensory impairment.

Reference fish held in contaminated lake water were similarly unable to respond to conspecific alarm cue.

Neurophysiological responses to standard olfactory cues (conspecific alarm cues and amino acids) appear to be different if fish are chronically or acutely exposed to metals. Although the mechanism responsible for this difference is not currently understood, ongoing experiments are currently being conducted to elucidate this phenomenon and to link neurophysiological responses to ecologically-significant behaviours.

Project Title:
(A2) Multiple metal interactions with fish gills and with natural organic matter

Investigator(s):
[1]Wilkie, M.P., [2]Dixon, D.G, [3]Borgmann, U., [1]Birceanu, O., [1,2]Winter, A.R., [4]Gillis, P.L., [1]Kara, Y., [4]Chowdhury, M.J., [1]McGeer, J.C., [4]Wood, C.M.

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Summary:
The Biotic Ligand Model (BLM) predicts how natural organic matter (NOM) and competing ions (e.g. Ca^{2+}, H^+, Na^+) affect metal bioavailability and toxicity in aquatic organisms, but does not consider metal mixtures. Two metals which may be co-released into aquatic ecosystems are Pb and Cd. Our first objective was to determine how exposure to environmentally relevant concentrations of Cd (< 100 $nmol \cdot L^{-1}$) plus Pb (< 500 $nmol \cdot L^{-1}$) affected metal-gill binding and gill-mediated ion (Na^+, Ca^{2+}) uptake in trout (*Oncorhynchus mykiss*). We predicted that in fish acclimated to soft water ($[Ca^{2+}]$~100 $\mu mol \cdot L^{-1}$), exposure to Pb plus Cd (without NOM), would lead to additive metal-gill binding because both metals target a single population of gill Ca^{2+}-channels. Surprisingly, we identified multiple populations of metal-gill binding sites. There was a high affinity, low capacity population of Pb-gill binding sites (apparent log $K_{Pb\text{-}gill}$=7.05; B_{max}= 18.2 $nmol \cdot g^{-1}$ gill), plus a low affinity population which could not be saturated. Two populations of Cd-gill binding sites were characterized: a high affinity, low capacity population (apparent log $K_{Cd\text{-}gill}$=7.33; B_{max}=1.73 $nmol \cdot g^{-1}$ gill), and a low affinity, high capacity population (apparent log$K_{Cd\text{-}gill}$=5.86; B_{max}=13.7 $nmol \cdot g^{-1}$ gill). At low concentrations, Cd plus Pb accumulation was less than additive because Cd out-competed Pb for binding sites, which were likely the Ca^{2+}-channels. This conclusion was supported by observations that Cd alone, but not Pb alone, inhibited Ca^{2+}-influx. In mixtures, however, Pb exacerbated Cd-induced disturbances to Ca^{2+}-influx in a more than additive manner (synergistic). Similarly, Pb alone, but not Cd, inhibited Na^+-influx by likely inhibiting the intracellular enzyme (carbonic anhydrase) that provides H^+ for the gill Na^+-H^+ transport system. However, Cd exacerbated the Pb-induced inhibition of Na^+-influx in a greater than additive manner. Our second objective was to determine how NOM affected metal-gill interactions during Pb-Cd mixture exposure. As expected, NOM reduced Pb-gill and Cd-gill binding when fish were exposed to each metal individually. Surprisingly, addition of NOM in the presence of Pb promoted Cd-gill binding, presumably because Pb out-competed Cd for NOM binding sites, which would have increased Cd bioavailability. We conclude that although Pb- plus Cd-gill binding is less than additive, the effects of such mixtures on branchial ion uptake, and possibly toxicity, are more than additive (synergistic). Moreover, by increasing the bioavailability of some metals, NOM may lead to greater metal-gill binding and potentially toxicity, when fish are co-exposed to NOM-metal mixtures. The authors gratefully acknowledge the support of the NSERC MITHE-Strategic Network.

Project Title:
(A8) The applicability of biotic ligand and critical residue approaches to Canadian Shield conditions.

Investigator(s):
[1]McGeer, J., [2]Vigneault, B., [1]Hicks, K., [1]AMancini, A., [3]Smith, S.

[1]Dept of Biology, Wilfrid Laurier University, Waterloo.
[2]Metals and the Environment Program, CANMET MMSL, Natural Resources Canada.
[3]Dept of Chemistry, Wilfrid Laurier University, Waterloo.

Summary:
The potential for metal impacts in aquatic systems depends on the geochemistry of the receiving environment as well as the sensitivity of the resident species. The biotic ligand approach is designed to predict impacts on a site specific water chemistry basis. Uncertainties in the application of the BLM for risk assessment include the variability in the complexation capacity of natural organic matters as well as differences in the sensitivity of native species. This project is addressing these uncertainties in the context of Canadian Shield waters. Studies on natural organic matter are focusing on four sources, three from soft water Shield lakes and one from a hard water lake. Complementary methods were used to characterize the interaction of Cu and NOM including: complexation capacity, UV absorbance, excitation-emission matrix spectroscopy and short term gill binding. Uptake of Cu to rainbow trout gills showed that concentrations of 10 mg/L DOC were equally effective in blocking accumulation. Free ion activity measurements demonstrated that the Cu complexation capacity of the NOMs from soft water were similar and higher than the hard water lake source. This similar complexation capacity for soft water sources occurred in spite of differences in their spectroscopic properties. The relative sensitivity of different species to Cu was tested by comparing acute toxicity responses in rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), brown trout (*Salmo trutta*), lake whitefish (*Coregonus clupeaformis)* and splake (*S. namaycush x S. fontinalis*). Waterborne Cu was highly bioavailable in soft water (Ca 40, Mg 20, Na 150, K 2 µmol/L, pH of 6.8 and dissolved organic carbon at <1.2 mg C/L) and EC50 values ranged from a low of 3.5 µg/L in brown trout to a high >22 µg/L for lake trout. To complement the acute toxicity data, short term Cu binding tests at similar Cu concentrations have been done. As expected from the BLM, Cu accumulation is inhibited by increased levels of Na^+. The protective effect of Na^+ varied among species but increased levels of Ca^{2+} in the exposure medium appeared not to reduce uptake. For both the species testing and the NOM characterization, measured data is compared to BLM predictions in order to understand the predictive capabilities of the model to Canadian Shield conditions.

Project Title:
(A9) Is the Biotic Ligand Model an appropriate model for multiple metal stressors?

Presentation Title:
Quantifying metal competition: Preliminary indications of the most appropriate parameters

Investigator(s):
Hoang, C.T.N., Hutchins, C., Mwakisenda C., England, R., Mandala, N., Simon, D., and Wilkinson, K.J.

Department of Chemistry, Université de Montréal, Montreal, QC

Summary:
The goal of this *recently undertaken* study is to rigorously test the Biotic ligand model (BLM) in soft waters containing metal mixtures that are typical of the Canadian Shield. Both bioaccumulation and sublethal toxicity are being monitored in short term experiments using *Chlamydomonas reinhardtii* as a test organism. Initial experiments have been designed to identify the best chemical and biological responses that will be employed to monitor competition under conditions that are relevant to Canadian Shield waters. In addition to bioaccumulation, genomic and some toxicological endpoints have been identified and tested. In addition, an equilibrium based resin technique has been set-up, optimized in laboratory and tested in the Rouayn-Noranda region. The measured signal increased in agreement with total dissolved metal concentrations but overestimated free ion concentrations. In the genomic assays, twenty genes have been identified where induction appears to be relatively specific for Cd (10) or Ni (10). The genomic signal, as evaluated by quantitative PCR, has now been optimized with respect to the exposure times. Further competition experiments are now underway in which a wider range of metals are being tested in order to isolate the genes which are the most specific with respect to Cd and Ni. In parallel, bioaccumulation experiments are being performed to determine to what extent the Biotic Ligand Model can be applied in the presence of multiple stressors (Cd+other trace metals; Ni+other trace metals). Initial results have indicated that for both Cd and Ni, the equilibrium based models should be appropriate for evaluating bioaccumulation. Finally, other parameters such as phytochelatin induction or measurements of photosystem II efficiency are now being optimized for use in the upcoming field studies (summer 2008).

Project Title:
(A4) Environmental risk assessment of metals in water and sediment: Importance of dietary uptake and water-sediment interactions to *Hyalella azteca*.

Investigator(s):
Last Name, initials (1st author); last name, initials (2nd author); etc.

Investigator(s):
[1]Dixon, D.G., [2]Borgmann, U., [1]Golding, L. and [1]Alves, L.
[1]University of Waterloo, Waterloo, ON
[2]Environment Canada, Burlington, ON

Summary:
DIETARY METAL TOXICITY. One of the most pressing questions currently facing aquatic environmental risk assessment is the degree to which metal accumulation from food contributes to, or is equivalent to, toxicity observed when the metal is accumulated from water. In other words, should dietary metal be considered in environmental risk assessment (ERA), assessment that is currently based almost exclusively on waterborne metal concentration. Our results indicate that bioaccumulation of Cd by *Hyalella* from contaminated field collected periphyton was much greater than from TetraMin fish food equilibrated with Cd in water. Toxicity was associated with Cd accumulated from natural periphyton, but we cannot determine if this was due to Cd, to other contaminants in the food, or to a reduction in food quality resulting from Cd and/or other contaminants. These experiments will be repeated using spiked periphyton where Cd is the only contaminant present and radio-labelled Cd will be used to quantify rates of uptake of Cd from TetraMin and periphyton.

SEDIMENT ERA. The second area of research focuses on the ERA of uranium in aquatic sediments. So far we have determined that Uranium toxicity is a function of how much U is bioaccumulated by *Hyalella.* Uranium bioaccumulation is predominately from the water, not the solid phase (sediment). Uranium uptake therefore involves a two step process: U dissociation from sediment and U uptake from water by *Hyalella*. Uranium dissociation from spiked sediment and U uptake from water by *Hyalella* can both be modelled using BLM type models. Both are primarily a function of pH. Ca^{2+} affects accumulation through its effect on speciation in water, not through direct competition for uptake of U. Future work will quantify U uptake kinetics and body size effects (if any), and measure toxicity and U bioavailability of field collected sediments to quantify U bioavailability under natural conditions.

Project Title:
(A5) Metal transfer along aquatic food chains

Investigators:
Hare, L, Campbell, P., Couture, P. and Fortin, C.
INRS-ETE, Université du Québec, Quebec City, QC

Summary:
The trace elements cadmium, nickel, selenium and thallium are accumulated by organisms and can thereby compromise their survival. Because the diet of aquatic animals can be an important source of trace elements, we are studying processes that control the uptake of these contaminants and their transfer along food chains.
Metal speciation. We measured the concentrations of major ions, trace metals and dissolved organic matter (DOM) in water from 18 lakes with wide ranges in pH, alkalinity, hardness and conductivity. Using 3D Excitation-Emission-Matrix fluorescence, we detected variations in the spectroscopic quality of DOM from the various lakes. We are investigating the influence of DOM quality on the speciation of Cd, Cu, Ni, and Zn in lakewater, and these metal speciation measurements will be compared with the predictions of chemical equilibrium models.

Algae. We completed measurements of intracellular Cd distributions and thiolated-peptide synthesis in two species of green algae. By measuring peptide synthesis at various Cd concentrations, we showed that algal species can differ widely in their metal detoxification strategies (such strategies influence metal transfer to their consumers). We have also expanded our algal studies to include another major group (diatoms) that will be used in continuous algal cultures (chemostats) to study the influence of chemical speciation on metal uptake and possible changes in Cd, Ni and Tl cellular distribution over time.

Invertebrates. Measurements of the distribution of Cd, Ni, Se and Tl in the cells of prey have allowed us to estimate their potential for causing toxic effects. Furthermore, we found that metal partitioning in prey determines in large part metal assimilation by predators. We also showed that Cd concentrations can differ widely among species of the same genus such that pooling species for environmental assessments could lead to erroneous conclusions. Lastly, we found that Ni concentrations in lakewater are correlated with those of a potential biomonitor for this metal (the insect *Chaoborus*).

Fish. Using fathead minnows, we determined that: waterborne Ni accumulation in embryos and larvae is quite rapid (days) and increases with increasing Ni concentrations in water; water and food contribute equally to Ni in fish; time to egg hatching (but not survival) decreases when fish embryos are exposed to high aqueous Ni concentrations; Ni, but not Tl, influences the routine metabolic rate of fish.

Project Title:
(A6) Impact of selenium on the aquatic biota in the prairie ecosystems

Investigator(s):
[1]Hontela, A., [1]Miller, L.L., [1]Rasmussen, J.R., [2]Palace, V.P., [3]Hu, X. and [3]Wang, F.
[1]Dept. of Biological Sciences, Univ. of Lethbridge, Lethbridge, Alta
[2]Centre for Environ. Res. on Pesticides, Fisheries and Oceans Canada, Winnipeg, MB
[3]Dept. of Environ., Geography, Dept. of Chemistry, Univ. of Manitoba, Winnipeg, MB

Summary:
Selenium (Se), an essential element, is toxic at concentrations slightly above those required for homeostasis. Irrigation, mining and combustion of coal are major sources of Se for the aquatic environment. An increase in Se bioavailability is among the emerging issues linked to climate change and the increasing demands for irrigation waters and coal. The major toxicity symptoms in fish are teratogenic deformities; however sensitivity to Se varies between species. Oxidative stress is a key mechanism of Se toxicity. While the effect of Se on reproduction in fish is known, effects on other systems, including stress responses and metabolism, received less attention.
The objectives of this study are to: 1) characterize Se exposure in aquatic ecosystems in a coal mining area in Western Canada, 2) provide data on the transfer of Se in the aquatic food chains, and 3) elucidate the mechanisms of Se toxicity in different species of fish.
***i) Field studies* - Coal mining region (Hinton, Alta)**
To follow up our previous studies in streams, four end pit lakes (2 reference, 2 Se-contaminated) near Hinton were stocked with hatchery rainbow and brook trout to initiate a large study that will provide new data on Se uptake by fish, transfer of Se in the food web, and effects of Se. Fish, invertebrates and water were sampled in summer and fall 2007 (time 0 and 4 months). Analysis of water Se and speciation confirmed the exposure gradient. Metabolic, hormonal, reproductive and oxidative stress endpoints will be measured in fish. The lakes will be sampled again in spring 2008, fall 2008, spring 2009 and fall 2009.
South Tobacco Creek (Miami, MB)
The STC watershed was sampled in April and July 2007, and higher Se concentrations (up to 12 μg/L) were found in April. The concentration rapidly decreased to 3.4 μg/L in July, consistent with the low concentrations observed in September and October 2007 (1.8-2.4 μg/L). This completes the study on the seasonal variation in the Se concentration in this watershed.
ii) Se mesocosm study at Delta Marsh (MB)
To further study the dynamics of Se in prairie waters, three mesocosms were set up at Delta Marsh in May 2007 and dosed with ^{82}Se(IV). Samples of surface water, sediment porewater, sediment cores, and plants were collected from the mesocosms and the pond as well. ^{82}Se was monitored to study the movement and diagenesis of “newly discharged” selenite; and ^{80}Se was monitored for the background correction. Data are being processed and will be included in a diagenetic model, which will be compared with the model constructed in 2004 for the natural system.
iii) Species-specific Se toxicity – comparison of rainbow trout and brook trout
In vitro studies – To elucidate the mechanisms underlying the differences in vulnerability to Se, cells of rainbow trout (Se-sensitive) and brook trout (Se-tolerant) are exposed to Se in vitro and indicators of functional integrity and oxidative status are measured. Validation experiments have been completed for rainbow trout, in vitro studies with brook trout cells are planned for winter 2008.

Project Title:
(A7) Food-chain transfer and effects of selenium in waterfowl

Investigator(s)
Petrie, S., Research Director, Long Point Waterfowl & Wetlands Research Fund
Badzinski, S. Scientist, Long Point Waterfowl & Wetland Research Fund
Belzile, N. Full Professor, Laurentian Univ., Dept Chemistry & Biochemistry
Chen, Y. Adjunct Professor, Laurentian University, Depart Chem & Biochem
Ware, L. M.Sc. Student, University of Western Ontario
Brady, C. M.Sc. Student, University of Western Ontario

Summary:
The combined continental population of greater scaup (*Aythya marila*) and lesser scaup (*A. affinis*) has declined continually since the mid-1980's. One hypothesis for the decline is that contaminant burdens acquired on wintering or staging areas are impairing reproduction or survival. We determined that selenium (Se) was the only substance present at sufficient levels that could impact health or reproduction of scaup using the lower Great Lakes (LGL). We found that greater scaup had higher Se concentrations than lesser scaup, possibly because large numbers overwinter on LGL and thus exposed to Se (foods, sediments, etc) for a longer period of time. Therefore, it is important to determine what effects elevated Se concentrations have on greater scaup wintering on the LGL. This aspect of our research investigates whether greater scaup wintering on Lake Ontario acquired Se concentrations high enough (≥10 ppm) to impact their body condition or health. During winter 2006 and 2007, we collected 73 birds from Hamilton Harbor – Lake Ontario. We then analyzed liver tissue, feather, and blood samples for Se and other contaminants, plus determined body condition (fat and protein) of each bird. We also made a biological assay measuring concentrations of malondialdehyde (MDA), a product of oxidative stress. In addition, we also examined birds for visual evidence of chronic impacts on health possibly due to elevated Se burdens. We found elevated (≥10 ppm) Se liver concentrations in 98.6% of birds sampled. However, we found no correlation between Se concentrations and body condition (fat or protein) of birds. We did not find evidence of Se toxicosis or Se-related health impacts nor a relationship between liver Se and MDA concentrations. Scaup likely acquire high levels of Se by eating Dreissenid mussels, filter-feeding organisms capable of bioaccumulating Se and other toxins. Dreissenid mussels we sampled, on average, had elevated Se concentrations throughout each winter. Although Se was elevated within the greater scaup we collected, those burdens did not appear to affect aspects of health assessed in this study. There are, however, many other aspects of health we did not evaluate that might be affected by increased Se burdens. Further, it is noteworthy that 93% of greater scaup had liver Se concentrations were below the 33 ppm that has been shown to cause chronic health effects in laboratory studies on mallards. It is conceivable that scaup with extremely high Se burdens might die or show altered behaviour and were thus underrepresented or not included in our study. We will be initiating a captive study in 2008 to determine how elevated Se burdens might affect scaup immune function, behavior, survival, and how they cope with cold-stress during the winter period.

Student Poster Abstracts

Project Title:
(A4) Importance of water-sediment interactions for *Hyalella azteca* exposed to uranium-spiked sediment and different overlying water chemistries

Investigator(s):
[1,2]Alves, L.C., [2]Borgmann, U. and [1]Dixon, D.G.

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[2]Aquatic Ecosystems Protection Research Division, Environment Canada, P.O. Box 5050, Burlington, ON

Summary:
This study exposed *Hyalella azteca* to uranium-spiked sediments (0 to 10 000 ug U/g dry weight) and five different overlying waters varying hardness and alkalinity independently. Water pH had a major effect on the dissolution of U from the sediment and U bioavailability and uptake to the *Hyalella*. Uranium desorption into the water increased with increasing pH, while U toxicity was higher in overlying waters of low pH. Calcium had a minor role on U uptake; primarily through its influence on U speciation. Experiments with caged animals demonstrated that U bioaccumulation and toxicity was mainly via the water rather than the sediment. Uranium bioaccumulation was a more reliable indicator of U toxicity than U concentrations in water or sediment. These water-sediment and water-bioaccumulation interactions were satisfactorily explained using saturation models.

Project Title:
Evolution of Ni and Cd intracellular distribution in chemostat cultured phytoplankton

MITHE-SN Research Project (A5): *Metal transfer along aquatic food chains*
P.I.: Landis Hare, *INRS-ETE*

Investigator(s):
Bernier, J., Fortin, C. and Campbell, P.G.C.

Institut National de la Recherche Scientifique, centre Eau, Terre et Environnement (INRS-ETE), Québec, QC

Summary:
It is well known that metal intracellular distribution influences metal trophic transfer to primary consumers. However, the intracellular distribution of accumulated metals within phytoplankton cells may vary with time as a result of detoxification mechanisms and thus alter trophic transfer efficiency.

Our objective is to determine how the intracellular distribution of Cd and Ni in phytoplankton cells changes as a function of the exposure time. The test species is the unicellular green alga *Chlamydomonas reinhardtii*. Algal cells have been cultured and exposed to metals in chemostat in order to provide a constant renewal of the exposure medium and thus minimizing metal depletion and other changes in chemical conditions within the exposure medium. Exposures at sublethal free ion concentrations of 10^{-7} M Ni^{2+} and 10^{-9} M Cd^{2+} for one, three and five days have been made. Cd^{2+} and Ni^{2+} concentrations have been monitored in the chemostat using an ion-exchange equilibration technique. Finally, the metal intracellular distribution has been examined by cell lysis (sonication) and subsequent differential centrifugation followed by metal burden quantification (ICP-MS) in the cellular fractions obtained.

Results show that the intracellular distribution of Cd and Ni does not significantly change after three days of exposure. However, Ni and Cd have different intracellular accumulation patterns. Ni accumulates more in cellular debris (*e.g.* membranes) than Cd, whereas Cd accumulates more in organelles and heat-stable proteins (*e.g.* phytochelatins) than Ni. Based on the fact that metals associated with cellular debris are less bioavailable for primary consumers, we can hypothesize that Ni is less bioavailable than Cd.

Project Title:
(A4) Metal bioaccumulation from food vs. water: A comparison of Cd bioaccumulation in *Hyalella* exposed to a field collected periphyton diet and lake water

Investigator(s):
[1,2]Golding, L., [2]Borgmann, U. and [1]Dixon, D.G.

[1]University of Waterloo, Waterloo, ON
[2]Environment Canada, Burlington, ON

Summary:
Determining the separate contributions of dietary and waterborne cadmium (Cd) accumulation in the freshwater amphipod *Hyalella azteca* is important for developing models that predict Cd bioaccumulation and toxicity. Such predictions can then be applied to metal contaminated sites for the purposes of risk assessment and setting site-specific water quality guidelines.

We conducted a 28 day laboratory-based experiment using water and periphyton collected from 3 lakes (Lac Opasatica, Lac Joannes, Lac Dufault) situated in the metal mining region of Rouyn-Noranda, Quebec, Canada. These lakes represented reference (0.089 nmol Cd/L), low Cd contaminated (0.61 nmol Cd/L) and high Cd contaminated (2.6 nmol Cd/L) aquatic ecosystems respectively. Cd bioaccumulation and toxicity in *Hyalella* were investigated using three exposure pathways: food-only (FO), water-only (WO) and food + water (FW). FO treatments consisted of field collected periphyton in a non-contaminated standard artificial medium, WO treatments consisted of a standard laboratory fish flake diet (TetraMin) with lake water. FW treatments consisted of field collected periphyton and lake water. Periphyton diets were apportioned on an equivalent ash-free dry mass basis relative to the TetraMin diet. Juvenile *Hyalella* were exposed for 28 days to the FO, WO and FW treatments concurrently and survival, dry mass and Cd bioaccumulation recorded. Water and diets were also analyzed for Cd over the exposure period.

Cd bioaccumulation in *Hyalella* increased with site contamination in FO, WO and FW treatments. In both the low and high Cd lake waters, there was a marked increase in Cd bioaccumulation when *Hyalella* were fed field-collected periphyton compared to the standard TetraMin diet. Water and dietary sources of Cd were incorporated into a prototype bioaccumulation model, that predicted as much as 71% of the Cd in *Hyalella* was attributable to Cd in the periphyton diet. Toxicity to *Hyalella* was observed with a significant reduction in survival in both the FO and FW treatments in the high Cd lake site affirming the influence of the natural periphyton diet common to both treatments. The dry mass of *Hyalella* in any treatment receiving periphyton for 28 days was significantly lower than *Hyalella* receiving TetraMin which indicates that differences in the nutritional quality of the periphyton and TetraMin diets may be an additional factor to metal contamination in affecting growth.

This research demonstrates that Cd is bioaccumulated by *Hyalella* from a living periphyton diet to a greater degree than a TetraMin diet. Initial model predictions suggest the proportion of Cd bioaccumulated in *Hyalella* from a periphyton diet is high relative to water. This model will be refined by incorporating kinetic data generated in future experiments. Lethal and sublethal toxicity is linked to the Cd contaminated periphyton diet but clarification of the role of the nutritional quality of the diet on toxicity is required.

Project Title:
(A8) Using biological and geochemical methods to characterize Cu complexation with NOM from different sources

Investigator(s):
[1] Hicks, K., [2]Vigneault, B., [1]McGeer, J., [1]Mancini, A., [3]Smith, S.

[1]Dept of Biology, Wilfrid Laurier University, Waterloo.
[2]Metals and the Environment Program, CANMET MMSL, Natural Resources Canada.
[3]Dept of Chemistry, Wilfrid Laurier University, Waterloo.

Summary:
This study examined the differences in Cu complexation among natural organic matter (NOM) sources from Canadian Shield waters. NOM samples were concentrated by reverse osmosis from 4 different sources. These sources include three soft water lakes (Brandy, Echo, and Nipissing) and one hard water lake (Bannister). Concentrating involved reducing approximately 200L of water to 4L. The concentrate was passed through a cation exchange resin to remove metal contamination. Complementary methods were used to characterize the NOMs and their interaction with Cu including, complexation capacity, UV absorbance, coloured fraction of DOC, excitation-emission matrix spectroscopy and short term gill binding. Complexation capacity was assessed directly by measuring free copper activity in spiked exposure media using an ion selective electrode. The coloured fraction of DOC and UV absorbance spectra were also measured to compare the composition of the different NOM tested in the gill binding experiments. To support the interpretation of the toxicity tests, gill binding assays were done with rainbow trout exposed for 6h to 16 µg Cu/L with 10 mg C/L of the 4 different NOM sources. All NOM sources similarly reduced the amount of Cu binding to the gill, suggesting no differences among NOMs. At a total copper concentration of 80 µg/L, the copper complexation capacity of Nipissing, Echo and Brandy were also similar. The complexation capacity of Bannister was however much lower. In addition, free activity measurement confirmed that the 24h equilibration time for the gill binding experiment was sufficient since no difference in copper complexation were observed between 24 and 48 h for any of the NOMs. The coloured fraction of DOC ranged from about 1 for Echo to 70 µg/L of quinine sulfate for Nipissing and therefore do not appear to be related to the copper complexation capacity. Similarly, differences in UV absorbance spectra at 10 mg C /L were observed for the different NOMs, but no clear relationship with the copper complexation capacity has been noted so far. NOM from Brandy and Bannister had very similar absorbance and had the highest absorptivities. NOM from Nipissing had intermediate absorptivities and Echo has the lowest absorptivities. No major differences were observed between the NOM regarding UV absorbance. Results will be discussed in the context of Cu speciation as predicted by the Biotic Ligand Model.

Project Title:
(A6) Biogeochemistry of selenium in the prairie waters in Southern Manitoba

Investigator(s):
Hu, X., Wang, F., Hanson, M.L.
Department of Environment and Geography, University of Manitoba

Summary:
Selenium is an essential micronutrient in animals and humans, though it can cause toxicity which is dependent not only on its concentration but also its speciation. Irrigation-dependant agriculture is wide spread in western Canada, and considered to be a possibly significant source of selenium in the prairie waters. As part of Project A6, the objective of this specific study is to assess selenium levels and speciation in wetlands in southern Manitoba, and to assess their response to further increases in the selenium loading.

Samples of surface water, sediment, sediment porewater, and aquatic plants were collected from three sites in southern Manitoba: Delta Marsh, Stephenfield Reservoir, and the South Tobacco Creek Watershed. The selenium levels in the surface water were relatively low (0.4-2.4 µg/L) compared with the water quality guidelines, but seasonal variation was observed in the South Tobacco Creek Watershed, where ~11 µg/L of selenium was found in the surface water during the spring freshet season.

A mesocosm study with selenite ($^{82}SeO_3^{2-}$) addition was conducted in Delta Marsh, Manitoba during the summer of 2007. By tracing the concentration of ^{82}Se in different selenium species in the surface water and sediment porewater, the transport and transformation of selenium in prairie wetlands were investigated. Preliminary results indicated rapid removal of Se from the surface water, and the Se speciation at Delta Marsh was dominated by an organoselenium species which is yet to be identified.

Financial support from the MITHE-SN and NSERC is gratefully acknowledged.

Project Title:
(A5) Metal transfer along aquatic food chains

Investigators:
Lapointe, D. and Couture, P.

Centre Eau, Terre et Environnement, Institut National de la Recherche Scientifique.

Summary:
Do nickel and thallium affect fathead minnow (*Pimephales promelas*) early-life stages?
In a previous experiment, we studied the relative importance of water and food as nickel (Ni) and thallium (Tl) sources for juvenile fathead minnow (FHM). We determined that both water and food contributed significantly to Ni accumulation but that fish exposed to waterborne Ni had significantly higher concentrations of Ni in metal sensitive fractions than control fish. We also determined that fish exposed to Tl from food or from water and food simultaneously had higher concentrations of Tl in metal sensitive fractions than control fish. Based on these findings, we hypothesized that waterborne Ni was more likely to be harmful to FHM whereas dietborne Tl constituted a greater threat.

Following that first experiment, we exposed FHM to environmentally realistic concentrations of Ni or Tl in six treatments: control, water only (low concentration), water only (high concentration), food only, water (low) + food and water (high) + food from the embryo stage and for 21 days. The objectives of this study were to determine (i) Ni and Tl accumulation in FHM embryos and larvae, (ii) the time to hatch and mortality rate in each treatment, (iii) the routine metabolic rate (RMR) and (iv) the activity of four key metabolic enzymes (CCO, LDH, NDPK and GST). In each treatment, organisms were sampled after 1, 2, 7, 14 and 21 days of exposure for metal analysis. Larvae were also sampled less than 24h post hatching. Mortality was recorded during the whole exposure. After 28 days of exposure, we measured the RMR on a sub-sample of each treatment. Preliminary results suggest that aqueous Ni exposure decreased the time to hatch but did not affect survival. We observed a merely significant effect of Ni, but not Tl, on RMR. Enzymes assays will be completed shortly. Preliminary results suggest that the level of contamination we used had little impact on early-life stage survival or metabolic rate.

Project Title:
Importance of metal detoxification strategies in explaining Cd resistance among phytoplanktonic species

MITHE-SN Research Project (A5): *Metal transfer along aquatic food chains*
P.I.: Landis Hare, *INRS-ETE*

Investigator(s):
Lavoie, M., Le Faucheur, S., Fortin, C. and Campbell, P.G.C.

Institut National de la Recherche Scientifique, centre Eau, Terre et Environnement (INRS-ETE)

Summary:
Within the MITHE strategic network, we are exploring the influence of inducible metal-binding peptides and intracellular metal partitioning on metal transfer from phytoplankton to herbivores. The importance of detoxification mechanisms such as metal sequestration in granules and thiolated peptides and their modulation under varying metal exposure conditions are not well known in microalgae and could affect metal transfer to herbivores in the food chain. A better understanding of these detoxification strategies among phytoplankton species is thus needed to enhance our knowledge on metal transfer in food webs. In this project, we studied the synthesis of thiolated peptides in connection with intracellular cadmium (Cd) distribution in two freshwater algae, *Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata*, exposed to a range of free Cd^{2+} concentration from 10^{-13} to 10^{-7} M in order to compare metal detoxification mechanisms among species. Five intracellular fractions (cellular debris, granules, organelles, heat-stables proteins (HSP), heat-denatured proteins (HDP)) were separated by differential centrifugation. Quantification of thiolated compounds such as phytochelatins and their precursors (glutathione and γ-glutamylcysteine) was done by HPLC with monobromobimane (mBBr) pre-column derivatization. Even though Cd uptake per cell was about two times higher for *C. reinhardtii* than for *P. subcapitata*, *C. reinhardtii* was more resistant to Cd with an EC_{50} of 133 nM Cd^{2+} compared to 43 nM Cd^{2+} for *P. subcapitata*. A significant increase in the proportion of Cd present in the granules fraction was observed for *C. reinhardtii* between 1 and 10 nM Cd^{2+} whereas no significant effect was obtained for *P. subcapitata*. Our results suggest involvement of granules in protecting against the toxic effects of Cd in *C. reinhardtii*. Both species produced high levels of phytochelatins but with longer oligomers for *C. reinhardtii*. Unknown thiolated compounds (X_n), which were not canonical or hydroxymethyl phytochelatins, were also found in both algae but in much larger quantities in *C. reinhardtii* than in *P. subcapitata*. This difference in thiol synthesis could also be involved in the higher Cd resistance of *C. reinhardtii*. This study confirms our initial hypothesis that metal partitioning and detoxification strategies vary among phytoplanktonic species which should in turn influence trophic transfer.

Project Title:
(A6) Influence of selenium on rainbow trout and brook trout inhabiting Se contaminated streams and lakes

Investigator(s):
[1]Miller, L.L., [1]Rasmussen, P., [2]Palace, J.R., [3]Wang, V.P., and [1]Hontela, A.

[1]Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta
[2]Centre for Environmental Research on Pesticides, Fisheries & Oceans Canada, Winnipeg, MB
[3]Department of Environment and Geography, and Department of Chemistry, University of Manitoba, Winnipeg, MB

Summary:
Selenium (Se) is an essential element that can bioaccumulate and become toxic at concentrations slightly greater than those required to maintain homeostasis. The major toxicity symptom in fish is teratogenic deformities; however, sensitivity to Se toxicity varies between species. The effect of Se on reproduction has been well studied, but Se's effect on other systems, such as the physiological stress response (PSR), has received less attention. The PSR enables fish to maintain homeostasis in response to a stressor and plays a role in the allocation of energy reserves. Previous work has shown that Se can activate the PSR of rainbow trout (RNTR) and increase plasma cortisol (the primary stress hormone in fish) and that at higher concentrations Se may also inhibit the release of cortisol from head kidney cells.

The objectives of this project are to compare the effect of Se on: (1) the PSR, (2) bioaccumulation of Se, (3) seasonal energy reserves, and (4) hepatic oxidative stress markers in RNTR and book trout (BKTR). Fish were collected from Se contaminated and reference streams near active open pit coal mines (study completed). Plasma (cortisol, glucose, T3, T4), liver (GSH, LPO, GPx), gill (Na^+/K^+ ATPase), muscle (Se) and head kidney (cortisol secretion) samples were collected and analyzed. Additionally, RNTR and BKTR were stocked into two Se contaminated and two reference end pit lakes and sampled (study ongoing). In addition to the samples described above, energy reserves (muscle glycogen and triglycerides in liver and muscle), stomach contents (diet, Se) and reproductive indices (GSI, maturity, plasma gonadotropin, plasma sex steroid hormones) will be assessed.

The PSR of fish exposed to Se in streams was not activated (plasma cortisol levels unchanged) or impaired (cortisol secretion unchanged). BKTR had lower hepatic GSH reserves than RNTR. Oxidative damage (liver LPO) increased with increasing Se exposure in RNTR, but decreased with increasing Se exposure in BKTR suggesting RNTR are more sensitive to Se induced oxidative stress. Preliminary results from the end pit lake study will also be discussed.

Project Title:
(A6) The effect of sodium selenite and selenomethionine on head kidney function in rainbow trout

Investigator(s):
Miller, L.L. and Hontela, A.
Department of Biological Sciences, University of Lethbridge, Lethbridge, AB

Summary:
Selenium (Se), an essential element, can be toxic a concentrations slightly greater than those needed to maintain homeostasis. The different forms of Se have different toxicities and selenite appears more toxic than selenomethionine *in vivo*. Cortisol, a steroid synthesized by the head kidney in teleosts, is synthesized in response to an internal or external stimulus the fish perceives as a stressor. It is responsible for a series of physiological changes that allow fish to maintain homeostasis.

The objectives of this study were to determine if (1) selenium compounds impair head kidney cell function and (2) sodium selenite is more toxic than selenomethionine to rainbow trout head kidney cells. Primary cultures of juvenile rainbow trout (*Onchorhynchus mykiss*) head kidney cells were exposed to sodium selenite or selenomethionine for one hour in a dose response study. Cell viability was assessed using a lactate dehydrogenase *in vitro* toxicity kit and trypan blue, while cell function was assessed by measuring cortisol secretion after a one hour stimulation with ACTH.

Exposure to sodium selenite inhibited cortisol secretion in a dose dependant manner, but did not alter cell viability. This suggests that Se, in the form of sodium selenite, can impair cortisol secretion. The effects of selenomethione on head kidney cell function and viability are under investigation. Results from these experiments can be used to predict Se toxicity in fish. *(Funded by MITHE-RN and Alberta Ingenuity)*

Project Title:
(A1) Generation & field-validation of chronic biotic ligand models for fish

Metal contamination impairs olfactory-mediated behaviours in wild fish: predator recognition, survival and recovery

Investigator(s):
[1,2]Reehan, S.M., [2]Patrick, R., [1,2]Warren, W.G., [1]Wood, C.M. and [2]Pyle, G.G.

[1]McMaster University, Hamilton, ON
[2]Nipissing University, North Bay, ON

Summary:
Recently, several studies have found that waterborne metal exposure (30 min – 7d) can impair olfactory capability in a variety of fishes resulting in the inhibition of olfactory-mediated behaviours important in survival and ecological functioning. Olfactory impairment is temporary and recovery can occur within 30 min to 7-10d (depending on length of exposure). However, previous studies have tested the inhibition of olfactory-mediated behaviours in laboratory or aquaculture-reared fishes without any direct evidence to demonstrate loss of ecological benefits. We conducted a series of studies in wild fathead minnows (*Pimephales promelas*) from clean and contaminated lakes to determine whether minnows could learn the identity of a novel predator and if predator recognition conferred a survival benefit upon encountering predators. In laboratory trials, bass naïve minnows from the clean lake learned the identity of previously unknown smallmouth bass, but bass naïve minnows from a contaminated lake did not. We also tested survival of minnows trained to recognize smallmouth during staged encounters with smallmouth bass. Minnows from the clean lake exhibited enhanced survival when trained to recognize bass compared to minnows not trained to recognize the bass. However, minnows from the contaminated lake exhibited the same level of survival irrespective of predator training. In a second study, we tested whether fathead minnows from a contaminated lake recovered olfactory-mediated behaviours after being transferred to clean water for either 7-12 or 30-37d using a reciprocal cross-exposure design. Minnows from either a clean or contaminated site were split into 2 groups and each group placed in either water from a contaminated or clean lake. After the exposure period minnows from each group were tested for their fright response to minnow alarm cues. Irrespective of exposure time, minnows from the contaminated lake did not respond to minnow alarm cue regardless of being held in clean or contaminated lake water. Minnows from the clean site responded to alarm cues if held in clean lake water, but exhibited a reduced fright response to alarm cues if held in contaminated water for 7-12d and no fright response after a 30-37d exposure. Collectively, our data demonstrates that minnows from contaminated lakes cannot use chemical information to learn the identity of unknown predators leading to an ecological loss of a survival benefit. Moreover, changes in water quality i.e., transfer to clean water, does not result in recovery of olfactory-mediated behaviour even after 30-37d. Environmental regulators need to consider the ecological functioning of aquatic organisms in addition to water quality.

Project Title:
Measured and modeled chemical speciation of Cd, Cu, Ni, and Zn in Canadian Precambrian Shield lakes and the influence of dissolved organic matter

(A5) Metal transfer along aquatic food chains

Investigator(s):

Mueller, K.K., Campbell, P.G.C., Fortin, C.

Institut National de la Recherche Scientifique, centre Eau, Terre et Environnement (INRS-ETE), Québec, QC.

Summary:
In aquatic systems, the speciation and geochemical mobility of many trace metals is influenced by dissolved organic matter (DOM). DOM also plays a key role in metal ecotoxicology by complexing the free metal ion; the free metal ion species best predicts metal bioavailability and hence toxicity. The binding of metal ions by DOM is thought to vary as a function of the "quality" of DOM. Our hypothesis is that the quality of DOM may be incorporated into chemical equilibrium models, such as the Windermere Humic Aqueous Model (WHAM), to improve speciation predictions for natural aquatic systems by taking into account variations in the metal complexation properties of DOM.

In situ diffusion samples were collected in triplicate from 18 lakes from the regions of Rouyn-Noranda, Quebec and Sudbury, Ontario, Canada. Each sample was analysed for water quality parameters such as pH, alkalinity, conductivity and the concentrations of major cations and anions. The total dissolved concentrations of Cd, Cu, Ni and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS). The free metal concentrations (M^{2+}) were determined using an ion exchange technique (Cd, Ni, Zn) or an ion-selective electrode (Cu). The concentration of DOM was also measured as dissolved organic carbon (DOC), while its quality was measured by both UV-visible absorbance and fluorescence. 3D-exciation/emission matrix (3DEEM) fluorescence spectra were analysed using the PARAFAC statistical analysis tool.

A large variability in water pH and DOC concentrations was observed. Variability was also observed in total dissolved Ni, Cu, Zn and Cd concentrations. The concentrations of Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} are currently being determined. With respect to the quality of DOM, the 3DEEM fluorescence spectra revealed visible differences. PARAFAC statistical analysis is used to identify the minimum number of components needed to explain the fluorescence spectra and to determine the relative proportions these components. Measured differences in the trace metal speciation in the lakes sampled will be related to water quality differences, including DOM quantity and quality. Ultimately, the DOM fluorescence characteristics will be used to estimate the "active" DOM fraction, i.e. the fraction which participates in metal complexation reactions. This DOM fraction will be incorporated into the chemical equilibrium model, WHAM, in order to better predict the speciation of environmentally significant trace metals in aquatic systems.

Project Title:
Monitoring nickel contamination in lakes using the phantom midge *Chaoborus*

(A5) Metal transfer along aquatic food chains

Investigators:
Ponton, D. and Hare, L.

INRS-ETE, Université du Québec, Quebec City, QC

Summary:
The determination of exposure in ecological risk assessments for metals is complicated by the fact that these contaminants exist as a number of chemical species such that total metal concentrations in lakewater tend not to correlate well with metal concentrations in aquatic animals. However, biological effects can be correlated with the concentration of the free metal ion, $[M^{2+}]$, provided that the concentrations of competing ions such as Ca^{2+} and H^+ are considered. Measuring $[M^{2+}]$ in lakewater is difficult, as is determining the influence of various ions competing for entry with M^{2+} at biological uptake sites. Thus metal measurements in animals have been proposed as a means of estimating bioavailable metal concentrations in their surroundings, that is, metal exposure.

We tested whether the phantom midge *Chaoborus punctipennis* could be used to monitor bioavailable nickel (Ni) concentrations in lakes. We chose this insect because previous studies have shown that it can be used to estimate cadmium exposure in lakewater and because it is very tolerant and thus likely to be found in even the most Ni contaminated and acidified lakes. We collected water and *C. punctipennis* larvae from 14 lakes located along a Ni gradient in the mining regions of Sudbury and Rouyn-Noranda. We measured pH, trace metals and major ions, as well as inorganic and organic carbon in lakewater for use in calculating ambient metal speciation (using the computer code WHAM; Windermere Humic Aqueous Model). Nickel concentrations in *C. punctipennis* varied widely among our study lakes, suggesting that this metal's availability to animals also varies in nature and that the animal thus has potential as a Ni biomonitor. To confirm that Ni concentrations in this biomonitor were indeed related to those in its surroundings, we compared Ni concentrations in our biomonitor to those in lakewater. The correlation between the two was significant but weak. We found that it could be much improved by considering the influence of potential competitors at biological uptake sites for Ni (e.g., H^+, Ca^{2+} and Mg^{2+}). Overall, our results suggest that *Chaoborus* larvae would make excellent biomonitors of Ni contamination in lakes and as such would be a useful component of risk assessment strategies designed to evaluate the hazard for aquatic organisms in lakes.

Title:
Why bother to identify animals used for contaminant monitoring?

(A5) Metal transfer along aquatic food chains

Investigator(s):
Proulx, I. and Hare, L.

INRS-ETE, Université du Québec, Quebec City, QC.

Summary:
Measurements of contaminants in animals are an important component of ecological risk assessments because they provide the link between contaminant exposure and toxicity.
In contaminant monitoring studies, pooling closely related species is a common practice because distinguishing species morphologically can either take too much time or be impossible. However, doing so, presumes that contaminant concentrations do not differ markedly among the pooled species. To test this assumption, we set out to determine if sympatric species of the genus *Chironomus* differ in their trace metal concentrations and, if so, why. To achieve this, we collected *Chironomus* larvae from lakes located along a metal-contamination gradient and identified species using a combination of molecular and morphological techniques. Once species were separated, we measured their metal concentrations. We discovered that within a given lake, species of *Chironomus* can differ widely in their Cd concentrations. Sulfur isotopic ratios revealed that these differences likely reside in the manner in which each species feeds on sediment. Overall, our results suggest that pooling *Chironomus* species could lead to erroneous conclusions about the Cd-contamination levels of lakes.

Project Title:
(A2) Investigating how natural organic matter influences metal gill binding when rainbow trout (*Oncorhynchus mykiss*) are exposed to metal mixtures.

Investigator(s):
[1,2]Winter, A.R., [1]Playle, R.C., [2]Dixon, D.G., [3]Borgmann, U. and [1]Wilkie, M.P.

[1] Department of Biology, Wilfrid Laurier University. Waterloo, ON
[2] Department of Biology, University of Waterloo, Waterloo, ON
[3] Environment Canada, Burlington, ON

Summary:
Playle (2004) used the toxic unit (TU) concept, where 1 TU is the 96 h LC50 for a particular toxicant (e.g. metal), to determine if metal-gill binding models such as the BLM could be used to predict metal-gill binding in fish exposed to multi-metal mixtures. An underlying assumption of the TU concept is that each metal would target the same population of gill binding sites and have the same toxic actions. Two metals that may be co-released into aquatic ecosystems are Pb and Cd, which target Ca^{2+} channels in the gill. Based on the TU concept, exposing fish to such metal mixtures should result in strictly additive toxic effects if the concentrations of the two metals sum to one toxic unit. Due to the non-linear nature of the metal-gill binding models, metal accumulation should be more than strictly additive below one toxic unit of metal exposure, and less than strictly additive (due to competition for binding sites) above one toxic unit. This research tested this model against reality by exposing juvenile rainbow trout (~1 g) to mixtures of Cd plus Pb. To investigate how Pb affects Cd binding to fish gills, trout were exposed to a range of water Cd concentrations (0.8, 1.5, 2.2 and 3.0 μM) while maintaining constant water Pb concentration (0.8 μM Pb). At the lowest water Cd concentrations (0.8 μM), Pb-gill and Cd-gill binding were approximately equal suggesting strictly additive metal-gill interactions at these relatively low Cd concentrations. Predictably, Cd-gill binding exceeded Pb-gill binding as water Cd was increased in the presence of Pb, but in a less than additive manner. When fish were exposed to Cd alone or Pb alone, the addition of 10 mg C/L natural organic matter (NOM) reduced both Cd-gill and Pb-gill binding, likely by reducing Cd and Pb bioavailability. However, during exposure to Cd plus Pb mixtures, the presence of NOM resulted in greater Cd-gill binding but lower Pb-gill binding. This affect was likely the result of Pb out-competing Cd for metal-NOM binding sites. We conclude the addition of NOM further complicates the interactions of Cd plus Pb mixtures with fish gills by likely increasing the bioavailability of Cd, but reducing Pb bioavailability. Further research should investigate how the presence of NOM alters the actual toxicity of Cd plus Pb mixtures to fish. The authors gratefully acknowledge the support of the NSERC MITHE-Strategic Network.

Reference: Playle, RC. 2004. Aquat. Toxicol. 67: 359-370.