Platform Presentation

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):
Dixon, D.G.[1]; Borgmann, U.[2]; Golding, L.[1]; and Alves, L.[1]; and Norwood, W.P.[2];
[1] University of Waterloo, Waterloo, ON
[2] Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, ON

Summary:
1. Toxicity and modeling bioaccumulation of cadmium from water and a periphyton diet.

The objectives of this research were to determine the relative contributions of Cd from water and diet to bioaccumulation in *H. azteca* and link this to toxic effects. Modeling and predicting toxicity from metal in dietary versus waterborne sources will assist in site specific risk assessments and development of protective aquatic guidelines.

Experimental results indicate that dietary Cd can compound toxicity from aqueous sources. Cd in diet contributed 84, 31 and 45% of the total bioaccumulated Cd in *H. azteca* in Cd-spiked periphyton-only, Cd-spiked water-only and Cd-spiked periphyton plus Cd-spiked water, exposures respectively.

2. Uranium toxicity, water-sediment and water-bioaccumulation interactions.

The first objective of this research was to determine U uptake kinetics and the effect of body size on U bioaccumulation and toxicity in soft water. Uranium bioaccumulation was significantly decreased with increasing body mass. Uranium was more toxic to juveniles than adults in one-week tests. A first-order saturation kinetic model was successful at predicting the uptake rate, elimination rate and the effect of gut-clearance on U body concentration in an acute and chronic test. The second objective of this research was to determine if water-bioaccumulation and water-sediment saturation models can quantify U bioavailability in natural waters and sediments collected near former uranium mining areas of Elliot Lake and Bancroft, Ontario, Canada. However, the water-bioaccumulation saturation model could not be fully validated since U concentrations in *H. azteca* exposed to contaminated water and sediments were similar to background levels. The water-sediment saturation model, nevertheless, under-predicted U concentrations in field sediments.

3. Metal Effects Addition Model (MEAM) Sub-project

A detailed risk analysis of 11 metals/metalloid at 32 MITHE-SN field sites is being conducted with *H. azteca* to validate the MEAM under natural conditions. Some interpretation of the presence and impact of an additional 16 elements in the sediment, water and tissue is also being evaluated. Thirty six *H. azteca* bioaccumulation/toxicity tests have been completed with a further 26 underway. In addition, three Se bioaccumulation/toxicity tests have also been completed as part of the development of a Se mortality model to be included in the MEAM.

Platform Presentation

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

Investigator(s):
Peter Campbell, Patrice Couture, Claude Fortin, Landis Hare (PI).
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 ranging widely in their pH, alkalinity, hardness and conductivity. For 5 of the 18 lakes, measured Cu^{2+} concentrations were in disagreement with those predicted by the WHAM VI geochemical speciation model. Furthermore, marked differences in DOM 3D-EEM fluorescence spectra were observed, both among lakes and between sampling regions. A possible relationship between the degree of metal complexation and the fluorescence quality of the DOM, as evidenced by fluorescence, is being investigated.

Algae. Algae exposed to sublethal concentrations of Cd^{2+} and Ni^{2+} for up to two weeks show variations in the manner in which these metals are distributed internally. Thus, between week 1 and week 2 there was a decrease in the proportion of Cd and Ni in the organelle fraction of *Chlamydomonas reinhardtii*, along with a concomitant increase in the proportion of these metals in the heat stable proteins fraction (*e.g.*, phytochelatins). These results suggest that algae used to assess metal transfer to a consumer would have to be exposed to Cd and Ni for a long period of time if such measurements are to be representative of metal transfer efficiencies likely to be obtained in the field.

Invertebrates. The concentrations of Cd and other metals vary widely among species of the insect *Chironomus* collected from lakes covering a wide range of sedimentary metal concentrations. Thus species can be characterised as being either hyper- or hypo-accumulators. Sediment metal concentrations are being measured to determine if this insect can be used to monitor sedimentary metals. In the water column, Ni concentrations in *Chaoborus* larvae varied widely among lakes, and were correlated with those in lakewater except in an acidic lake where [Ni^{2+}] were high and yet larvae accumulated little Ni. By considering Ni^{2+}-H^+ competition on *Chaoborus* (our laboratory experiments confirm that this insect takes up Ni mainly from water), we were able to explain this apparent outlier. Our results suggest that *Chaoborus* could be used to estimate water column Ni^{2+} concentrations in lakes.

Fish. In juvenile fathead minnows fed with the worm *Tubifex tubifex*, the proportion of Ni bound to heat-stable proteins and cellular debris was higher and the proportion of Ni bound to granules was lower than in fish fed with the insect *Chironomus riparius*, regardless of the level of contamination of their prey. Because we obtained a low level of metal bioaccumulation by *C. riparius*, especially for Tl, we investigated the trophic transfer of Ni and Tl using *Daphnia magna* and we compared these results to those we obtained for the two other prey types.

Platform Presentation

Project Title:
(A6) Impact of Selenium on the Aquatic Biota in the Prairie Ecosystems

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

Summary:
Selenium (Se), an essential element, is toxic at concentrations slightly above those required for homeostasis. Teratogenic deformities are among the symptoms of Se toxicity in fish; differences in sensitivity among fish species exist. While the effect of Se on reproduction in fish is known, effects on other systems, including stress responses and metabolism, received less attention. Coal and uranium mining, combustion of coal, and irrigation of Se-rich soils are major sources of Se for the aquatic environment. Increased bioavailability of Se in the aquatic environment may occur as a result of increased demand for coal, driven by the energy crisis, and increased demand for irrigation waters, as climate changes and water resources are threatened.
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. This project generates data on Se in fish tissues, in invertebrates consumed by the fish, in water, sediments, and sediment pore waters.
i) Field studies
Coal mining region (Hinton, Alta)
To compare the sensitivity of rainbow trout (RNT) and brook trout (BKT) to Se, four end pit lakes (two low Se, two high Se) situated in a coal mining area were stocked with hatchery RNT and BKT in summer 2007. Fish were sampled at time 0, 6, 12 and 18 months of exposure. A suite of morphological (condition, growth, gonad size), biochemical (liver GSH, LPO, glycogen) and physiological (plasma cortisol, T3, T4, reproductive hormones, liver and muscle glycogen and lipid content) endpoints are measured. To characterize exposure, muscle and gonads are analyzed for Se, along with stomach contents (invertebrates), lake water and sediments. The results indicate that RNT and BKT differ in the growth patterns, energy metabolism and oxidative stress responses, and responses to Se.
ii) Modeling
Data from the southern Manitoba sites were used as the input to produce a model describing the cycling of Se in the prairie waters. The model focuses on the concentration and speciation of Se in the surface waters and in sediments and their response to further input of selenite. The analysis suggested that the concentrations of total Se and selenite in the surface waters will remain low at the current level of agricultural activities in southern Manitoba. However, elevated Se is expected at the spring freshet season at some sites and if the irrigation activities continue to increase.
ii) Species-specific Se toxicity – comparison of rainbow trout and brook trout
In vitro studies – Vulnerability of fish cells (head kidney cells) to Se was compared in RNT (Se-sensitive) and BKT (Se-tolerant), using indicators of functional integrity (cortisol

production, viability) and oxidative status (GSH, lipid peroxidation, GPx,). Our results indicate that function of RNT cells is disrupted at concentrations of Se that are significantly lower than those causing cell death. Moreover, toxicity of two Se species was assessed in vitro; sodium selenite was more toxic than sodium methionine. In vitro studies with BKT cells are planned for 2009. The experiments will provide comparative toxicological data for two salmonid species.

Whole animal exposures to waterborne Se – lab. exposures with BKT are planned for 2009, to complement earlier studies with RNT.

Platform Presentation

Project Title:
(A6) Understanding Selenium Speciation and Behavior in the Aquatic Ecosystems of the Canadian Prairie

Investigator(s):
Xiaoxi Hu[†], Feiyue Wang[†§], and Mark L. Hanson[†]

[†] *Department of Environment and Geography,* [§]*Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada*

Summary:
Selenium contamination and impacts in aquatic ecosystems can occur in areas of high irrigation that are coupled with underlying seleniferous geology. The Canadian Prairie, much of which is underlain by seleniferous shale, has witnessed a significant expansion of the acreage under agricultural irrigation in recent decades. Hence, we hypothesize that areas of increased agricultural activity should exhibit elevated levels of selenium relative to native ecosystems in this region. To test this, we examined the selenium concentrations in surface water, sediments and porewater, and aquatic plants in southern Manitoba, Canada, from a number of representative aquatic systems. In addition, a high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) method was developed for determination of selenium speciation in natural waters. It allows for the isocratic separation of selenite, selenate, and several organoselenium species with no sample pretreatment required. Finally, a mesocosm study was performed to assess the transport and transformation of newly added selenium in a prairie wetland. Overall, our results indicate that selenium concentrations in the prairie waters of southern Manitoba are low at present and are dominated by selenate, and if selenium is discharged as selenite, it will be quickly removed from the surface water. However, selenium can be elevated during the snowmelt season and in areas of intensive agricultural activities. A probabilistic risk assessment for the region shows that the risk to aquatic organisms from current selenium concentrations and speciation is considered low.

Platform Presentation

Project Title:
(A7) Food-chain transfer and effects of Se in waterfowl: Speciation of organo Se compounds.

Investigator(s):
Petrie, S., Belzile, N., Badzinski, S., Chen, Y., Brady, C., Hontela, A, and Schummer, M.

Summary:
The primary purpose of this research is to determine if selenium acquisition is impacting (survival, condition, oxidative stress, immune response, etc.) diving and seaducks that stage and winter on the lower Great Lakes and to investigate the speciation of Se compounds in various tissues and aquatic environments. During 2008, A7 researchers have concentrated on 4 research areas. 1/ Analysis of scaup liver and egg samples collected at the Yukon-Kuskokwim Delta, Alaska, showed that some females initiate nesting with elevated hepatic concentrations of Cd, Hg, and Se and transferred Hg and Se to eggs, but adverse effects on condition and productivity are unlikely. 2/ Tissues of 3 species of diving ducks collected throughout winter on Lake Ontario were analyzed. All species accumulated selenium throughout winter. Overall, hepatic selenium concentrations were elevated (>10 ppm dw) in 99%, 58%, and 78%, of long-tailed duck, common goldeneyes, and buffleheads, respectively. It is unclear at present if acquisition of selenium concentrations of these magnitudes affects the short- or long-term health or reproduction of ducks that winter at Lake Ontario. 3/ A masters project was initiated to determine if survival and health of captive scaup are differentially affected by accumulation and chronic exposure to selenium (via dietary intake) during a six-month over-wintering period in Southern Ontario – captive work will be complete in March 2009. 4/ A forth project was initiated to develop analytical methods which will enable the highest organic Se extraction from a solid biological sample and the quantitative determination of the most common encountered Se metabolite (selenocystine Secys2, methylselenocysteine MeSeMet, selenomethionine SeMet, trimethylselenonide TMSe, etc.): (1) searching for effective digestion methods to completely recover organic Se compounds from solid biological sample; (2) investigate the optimal chromatographic conditions; (3) working on the inter phase connection between HPLC–UV irradiator–AFS. To date, several extraction systems have been tested and compared and more than 90% recovery of Se in liquid has been achieved. A simple protocol for TMSe synthesis has been established and purity analysis of this compound is satisfactory. Chromatographic conditions have been tested with the 4 mixed standard organic Se compounds and a better separation system identified. The next step is to test real sample extracts.

Poster

Project Title:
(A1) Generation and field validation of chronic Biotic Ligand Models for fish

Investigators:
Pyle, G.G. (Dept. of Biology, Lakehead University); Wood, C.M. (Dept. of Biology, McMaster University)

Summary:
Our work over the past year focused on four specific aspects related to the development of a chronic, chemosensory-based Biotic Ligand Model in fathead minnows, as follows: (i) examining temporal effects of dissolved Cd on chemosensory toxicity, (ii) establishing the effects of dissolved Na and DOC on Cu binding to olfactory epithelium (OE), (iii) relating Ca effects on Cu-OE binding dynamics to neurophysiological and behavioural responses to standard chemosensory stimuli, and (iv) determining the protective effects of DOC against Cu-induced chemosensory-mediated behavioural deficits.

Although previous research has demonstrated chemosensory dysfunction in rainbow trout exposed to 2.5 μg/L Cd, we have been unsuccessful in observing similar effects in fathead minnows. We exposed fathead minnows to 2.5 μg/L of dissolved Cd and tested their olfactory acuity after 3, 24, 48, 96, and 192 h of exposure using electro-olfactography (EOG) and behavioural approaches. However, both behavioural and EOG responses to standard chemosensory stimuli were indistinguishable from controls. These results suggest that 2.5 μg/L of dissolved Cd—although significantly higher than concentrations measured in metal-contaminated lakes in Ontario and Quebec—is insufficient to cause chemosensory toxicity in fathead minnows, but not in rainbow trout, and increasing exposure time does not appear to have an effect.

Dissolved organic carbon was effective at inhibiting Cu binding to fathead minnow olfactory epithelium, but dissolved Na was not. This result reveals that DOC is similarly protective against Cu-OE binding as it is against Cu-gill binding. Moreover, the reduction of Cu bound to the OE from DOC was related to protected olfactory function in Cu-exposed fish as determined by EOG and behavioural assays. At very low Cu concentrations (157 nM), DOC was *probably* still protective against Cu-OE binding, especially given the protective effects against olfactory toxicity observed in EOG and behavioural assays. However, the low Cu-exposure concentration and small tissue size resulted in analytical difficulties that warrant caution in the interpretation of our results.

In our previous work, we demonstrated that increasing Ca is effective at inhibiting Cu binding to fish OE, but only at Cu concentrations above 157 nM owing to cooperative Cu-OE binding. We exposed fathead minnows to 157 nM Cu for 3 h, which was sufficient to impair chemosensory function in animals held in soft water. When experimental fish were exposed to 157 nM Cu in water having increasing Ca concentrations (up to 1000 mM), olfactory function was not protected, suggesting that Cu and Ca may be binding to two different sets of binding sites in the OE. Taken together, these results suggest that there are inherent differences between gill and olfactory epithelium with respect to metal-binding dynamics that should be taken into account when developing a chemosensory-based BLM.

Poster

Project Title:

(A2) Differences in Natural Organic Matter (NOM) Quality Have Little Influence on Cu-Gill Binding in Rainbow Trout.

Investigator(s):

[1,2]Gheorghiu, C., [1]Wilkie, M. P. and [2]Smith, D.S.

[1]Department of Biology, Wilfrid Laurier University, Waterloo, ON. N2L 3C5.
[2]Department of Chemistry, Wilfrid Laurier University, Waterloo, ON. N2L 3C5.

Summary:

The Biotic Ligand Model (BLM) relates acute toxicity (LC_{50}) to the critical metal accumulation (LA_{50}) at the biotic ligand (e.g. fish gill). The BLM not only considers water chemistry (e.g. hardness, alkalinity, pH) but also complexation of metals by inorganic and organic ligands (natural organic matter; NOM) in natural waters, which decreases metal bioavailability. NOM is derived from external sources to the aquatic system (allochthonous), or it is generated within the water column (autochthonous). Allochthonous NOM is thought to be more protective against metal toxicity due to its greater metal binding capacity. However, the protective effects of different NOM may also result from differences in NOM quality, which can be measured using the specific absorbance co-efficient (SAC). It has been suggested that more darkly coloured NOM (allochthonous), with a higher SAC, is more protective than lightly coloured NOM (autochthonous).

Our objective was to determine if it were necessary to develop a quality factor for NOM that could be incorporated into the BLM. To achieve this goal, three different types of NOM (allochthonous, autochthonous and sewage derived) were first characterized using fluorescence spectra analysis, followed by determinations of Cu-gill binding when fish were exposed to comparable concentrations of each NOM type. As predicted, fluorescence spectra analysis showed that the molecular nature of the three NOMs were significantly different from one another.

After determining the 96-h LC50 for Cu in ion-poor water (10.5 $\mu g L^{-1}$/ 164.8 nM), we then exposed fish to a range of Cu concentrations in the absence or presence of 4 mg C L^{-1} of each NOM type examined. Our findings indicated the existence of both a low capacity, high affinity Cu-gill binding site population (B_{max} = 23.7 nmol g^{-1}; log $K_{Cu\text{-}gill}$ = 7.89), and a higher capacity, lower affinity population of Cu-gill binding sites which could not be saturated. At the copper concentrations tested, all NOM sources showed no differences in Cu-gill binding; all NOM isolates brought Cu accumulation at the gills down to background levels. We conclude that it is the amount, rather than the quality, of NOM that is most reliable predictor of Cu-gill binding in trout. The authors gratefully acknowledge the support of the NSERC MITHE-Strategic Network.

Poster – Student Competition

Project Title:
(A2) A Case of Metal-Mixture Synergy: Exacerbation of Cd-Induced Ionic Disturbances by Pb.

Investigator(s):
Kara, Y., Wilkie, M.P.
Department of Biology, Wilfrid Laurier University, Waterloo, ON.

Summary:
Traditionally, the majority of metals toxicity research has focused on individual metals rather than metal mixtures which are more likely to occur in contaminated waters. Moreover, little is known about the effects of metals on fishes living in the soft slightly acidic waters typical of the Canadian Shield. Our goal was to determine how mixtures of Pb plus Cd interact to exert their toxic effects at the fish gill. Previously, Birceanu et al. (Aquat. Toxicol. 89:222) reported that acute exposure (3h) to Pb plus Cd mixtures inhibited both gill Na^+ and Ca^{2+} uptake in a greater than additive (synergistic) fashion. We therefore hypothesized that these disturbances to gill ion uptake would result in greater than additive effects on internal ion balance and greater toxicity during longer-term Pb- plus Cd-mixture exposure.

Rainbow trout (*Oncorhynchus mykiss*) were fitted with surgically implanted cannulae to measure plasma ions (Ca^{2+}, Na^+, Cl^-), and other sensitive indices (pO_2, pH, Ht, Hb, protein, lactate) during longer term (5d) Pb plus Cd exposures in soft (Ca^{2+} ~ 100 µmol L^{-1}) , acidic (pH 6) water. Exposure to Cd only (10nM) caused a 23% reduction in plasma Ca concentration after 1d, followed by a recovery of plasma Ca^{2+} balance by 3d. No significant effects of Cd on blood chemistry were observed. In contrast, Pb only (50nM) had no effect on the measured blood parameters. However, the mixture of Pb (50nM) plus Cd (10nM) caused a substantial 26% decrease in plasma calcium after 1d, which persisted over the 5d period. Percent survival (16%) was also substantially lower during Pb + Cd exposure, compared to exposure to Cd (40%) and Pb (71%) individually. Thus, the combined physiological and toxic effects of Cd plus Pb exposure were greater than additive (synergistic). In contrast, the metal-gill burden revealed that exposure to Cd plus Pb mixtures appeared to cause less than additive metal-gill binding.

We conclude that exposure to Pb plus Cd mixtures at environmentally relevant concentrations results in synergistic toxicological effects, despite less than additive Cd- plus Pb-gill binding. The possibility of synergistic actions of metal mixtures may need to be considered in future environmental risk assessments and the future establishment of water quality criteria. The authors gratefully acknowledge the support of the NSERC MITHE-Strategic Network.

Poster – Student Competition

Project Title:
(A4) Uranium bioavailability, water-sediment and water-bioaccumulation interactions to Hyalella *azteca* exposed to sediment and site water collected near former uranium mining districts: Elliot Lake and Bancroft, Ontario, Canada

Investigator(s):
Alves, L.C.[1, 2], Borgmann, U.[2], Dixon, D.G.[1]
[1]Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, Canada
[2]Aquatic Ecosystems Protection Research Division, Environment Canada, P.O. Box 5050,Burlington, ON L7R 4A6, Canada

Summary:
The purpose of this study was to determine if a water-bioaccumulation and water-sediment saturation model developed in this lab using U-spiked water and sediment could be applied to quantify U bioavailability in natural water and sediments. U concentrations in *Hyalella azteca* exposed to contaminated water and sediments were at or close to the background levels for *Hyalella* exposed to the water and sediments collected from references sites near Elliot Lake and Bancroft and in controls from previous lab studies. Hence, the water-bioaccumulation saturation model could not be fully validated in this particular study. The water-sediment saturation model, however, on average under-predicted U concentrations in the sediment when compared to the observed field concentrations. Although, U speciation in the sediment was not measured, U may have been in the U (IV) form which has a strong tendency to precipitate and remain immobile in the sediment. Other factors such as organic carbon and the clay composition of the sediments could also have contributed to the higher U concentrations in the sediments, while limiting U bioavailbility more so than the overlying water chemistry. Overall, U in the water was too low to be toxic to the *Hyalella.* Uranium concentrations for *Hyalella* exposed to site soft waters were on average 90, 50 and 100 times lower than the estimated mean LBC10 in the *Hyalella* (29 nmol/g), LC10 in the water (72 nmol/L) and LC10 in sediment (15188 nmol/g), respectively, for animals exposed to similar artificial overlying soft-waters in previous lab studies.

Poster – Student Competition

Project Title:
(A4) Kinetics of uranium uptake in soft water and the effect of body size on bioaccumulation and toxicity to *Hyalella azteca*

Investigator(s):
Alves, L.C.[1, 2], Borgmann, U.[2], Dixon, D.G.[1]
1Department of Biology, University of Waterloo, Waterloo, ON, N2L 3G1, Canada
2Aquatic Ecosystems Protection Research Division, Environment Canada, P.O. Box 5050, Burlington, ON L7R 4A6, Canada

Summary:
This study evaluated the kinetics of uranium (U) uptake and the effect of body size on U bioaccumulation and toxicity to the freshwater amphipod, *Hyalella azteca,* exposed to water-only U concentrations in soft water for 7 days. The effect of body size on U bioaccumulation was significant, with a slope of –0.35 between log body concentration and log body mass. The one-week lethal-water concentrations causing 50% mortality based on a saturation model for juvenile and adult *Hyalella* were 1100 and 4000 nmol U/L, respectively. The one-week lethal body concentrations causing 50% mortality were 120 and 180 nmol U/g for the juvenile and adult *Hyalella*, respectively. A first-order saturation kinetic model was used to predict the uptake rate, elimination rate and the effect of gut-clearance on whole U-body concentration in *Hyalella* exposed to acute (7 days) and chronic (28 days) waterborne U concentrations. The effect of gut-clearance on total whole-body concentrations in the *Hyalella* was substantial, with an approximate 72-79% loss of total U-body concentrations in animals depurated for 24h.

Poster – Student Competition

Project Title:
(A4) Toxicity and modelling bioaccumulation of cadmium from water and a periphyton diet in the freshwater amphipod *Hyalella azteca*

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

Summary:
The objectives of this research were to determine the relative contributions of Cd from water and diet to bioaccumulated Cd in *Hyalella* and link this to toxic effects. Being able to model and predict toxicity from metal in dietary versus waterborne sources will assist in site specific risk assessments and development of protective aquatic guidelines.

We conducted a 28 d experiment consisting of Cd uptake from water and dietary pathways separately and combined. Juvenile *Hyalella* were exposed to Cd spiked periphyton (250 – 16000 nmol/g) and water (5 – 85 nmol/L) spanning low environmentally relevant and high lethal concentrations. Periphyton was used in the food-only and food+water exposure pathways while a commercial fish flake diet (TetraMin®) was used for Cd exposure via the "water-only" pathway. Bioaccumulation of Cd, growth, survival, total lipid and total protein of *Hyalella* were recorded.

Lethal body concentrations (LBC25) were measured at 252, 397 and 495 nmol Cd/g in periphyton-only, periphyton+water and water-only exposures respectively. Lethal aqueous concentrations (LC25) were measured at 1.3, 9.6 and 13.9 nmol Cd/L periphyton-only, periphyton+water and water-only exposures respectively. These results show how dietary Cd can compound toxicity from aqueous sources.

Non-linear regression modelling predicted that Cd in diet contributed to 84, 31 and 45% of the total bioaccumulated Cd in *Hyalella* in the periphyton-only, water-only and periphyton plus water exposures respectively. We hypothesize that when dietary and aqueous Cd concentrations are in equilibrium, diet contributes markedly to bioaccumulation but this diminishes as aqueous Cd increases. Under these circumstances, mortality associated with dietary Cd would not be expected to occur.

Poster

Project Title:
(A4) Metal Effects Addition Model (MEAM) Testing and Risk Assessment of MITHE-SN Aquatic Ecosystems Theme Sites. 2008/09 Sub-project of (A4) Environmental risk assessment of metals in water and sediment: Importance of dietary uptake and water-sediment interactions to *Hyalella azteca*

Investigator(s):
Norwood, W.P.[1]; Milne, L.A.S.[2,1]; Dixon, D.G.[2]

[1] Aquatic Ecosystem Protection Research Division, Environment Canada, Burlington, Ontario L7R 4A6.
[2] University of Waterloo, Waterloo, Ontario N2L 3G1

Summary:
The bioaccumulation and test water concentrations of As, Cd, Co, Cr, Cu, Mn, Ni, Pb, Se, Tl and Zn are used to predict toxicity (survival) of the test organism, *Hyalella azteca*. The predicted values are compared to the observed sediment toxicity test results in an evaluation of the Metal Effects Addition Model (M.E.A.M.). The M.E.A.M. was developed to predict the impact mixtures of metals on the benthic invertebrate, *H. azteca* based on bioaccumulated metals (Norwood, 2008). This model was developed as part of the Metals in the Environment Research Network (MITE-RN, 1999-2004). The MEAM is based on laboratory experimentation only, therefore, field validation of the method is required to demonstrate its use for environmental risk assessment. Field validation provides; biotic uptake and accumulation data from natural mixtures of metals in sediment and water (potential for trophic transfer), predictions of toxicity, and possible identification of metal-metal interactions that modify metal bioavailability outlined by Norwood et al. (2007).

The accumulation and toxicity of metals and a metalloid from MITHE-SN field site sediments and waters will provide detailed risk analysis of 11 metals/metalloid, across multiple lakes of varying water chemistry, using one method with one organism (*H. azteca*). As well, some interpretation of the presence (contamination levels and bioaccumulation) and impact of an additional 17 elements will be made. Thirty six sediment and water samples have been collected from 23 MITHE-SN sites across Canada. Thirty six *H. azteca* bioaccumulation & toxicity tests have been completed with a further 26 will conducted in 2009. ICP-MS analyses by the National Laboratory for Environmental Testing (Environment Canada, Burlington) of 27 elements in sediment, water and tissue samples will be conducted. Three Se bioaccumulation & toxicity tests have also been completed as part of the development of a Se mortality model to be included in the M.E.A.M.

Poster

Project Title:
(A5) Evolution of Ni and Cd intracellular distribution in chemostat cultured phytoplankton

Investigators(s):
Bernier, J., Fortin, C. and Campbell, P.G.C.
INRS-ETE, Université du Québec, Quebec City, 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 exposure time as a result of detoxification mechanisms and thus may 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 exposure time. The test species is the unicellular green alga *Chlamydomonas reinhardtii*. Algal cells have been cultured and exposed to metals in continuous cultures (*i.e.* in chemostats) in order to provide a constant renewal of the exposure medium and thus minimize metal depletion and other changes in chemical conditions within the exposure medium. Cells have been exposed to sublethal free ion concentrations of 10^{-7} M Ni^{2+} and 10^{-9} M Cd^{2+} for 15 days. 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.

Although free Ni^{2+} and Cd^{2+} ion concentrations decreased very rapidly by about 50 % and 99 % respectively, thereafter the exposure conditions stayed quite stable over 15 days. Intracellular distribution results showed, for both Ni and Cd, a statistical decrease of metal percentage within the organelle fraction between days 7 and 15 associated with a concomitant increase of the metal percentage within the heat-stable protein fraction (*e.g.* phytochelatins). This could result from the induction of detoxification mechanisms by acclimation. From an ecological risk assessment point of view, the use of short-term metal exposed algae may be appropriate to evaluate diet-borne metal bioavailability to primary consumers in pulse metal contamination scenarios. However, since metal intracellular distribution could vary over a prolonged exposure period, chronically exposed algae should be used for normal ERA scenarios (long-term, chronic exposure to metals).

Poster – Student Competition

Project Title:
(A5) Influence of prey type on nickel and thallium accumulation and toxicity in juvenile fathead minnows (*Pimephales promelas*).

Investigator(s):
Dominique Lapointe, Sophie Gentès, Dominic Ponton, Landis Hare & Patrice Couture
INRS-ETE, Université du Québec, Québec, QC.

Summary:
We previously investigated the relative contribution of water and prey as sources of nickel (Ni) and thallium (Tl) for larvae and juvenile fathead minnows (*Pimephales promelas*). While both metals were principally accumulated from water by larvae (exposed for up to 21 days), prey significantly contributed to Ni and Tl accumulation in juveniles. Thus, we set out to determine if feeding on prey having different patterns of Ni and Tl subcellular distributions would result in different assimilation efficiencies (AE), subcellular distribution and metabolic capacities in juvenile fathead minnows. We contaminated two prey types, *Tubifex tubifex* (a benthic oligochaete) and *Daphnia magna* (a planktonic crustacean), with either Ni or Tl and fed them daily to juvenile fathead minnows for 7 days. In *T. tubifex*, most of the Ni was bound to heat-stable proteins (HSP) and organelles whereas in *D. magna*, Ni was principally found in the HSP and granule-like fractions (trophically available Ni in the two prey types was estimated to be 85% and 51%, respectively). Tl was almost equally distributed among the granule, cellular debris, heat-denatured proteins (HDP) and HSP fractions in *T. tubifex* but was mostly bound to HSP and cellular debris in *D. magna* (trophically available Tl was estimated to be approximately 50% of the total in both prey types). We determined that Ni AE was approximately 10% regardless of prey type, whereas Tl AE was approximately 31% and 70% when fish were fed *T. tubifex* or *D. magna*, respectively. In fish, most of the Ni was found in the HSP and granule-like fractions, regardless of prey type. Fish fed *T. tubifex* had significantly higher proportions of Tl bound to the cellular debris and HDP fractions and significantly less Tl bound to HSP than fish fed *D. magna*. Neither prey type nor metal had a significant effect on fish routine metabolic rate.

Poster – Student Competition

Project Title:
(A5) Role of Ca and essential micronutrients in modulating Cd uptake and toxicity in phytoplankton

Investigator(s):
Michel Lavoie, Claude Fortin and Peter G.C. Campbell
INRS-ETE, Université du Québec, Quebec City, QC.

Summary:
Within the biotic ligand model (BLM), major cations such as Ca^{2+} are considered simple competitors for Cd^{2+} uptake into algal cells that will offer some protection against Cd-induced toxicity. The role of essential micronutrients (Fe^{3+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}) is considered negligible. However, recent studies in our laboratory using the green algae *Pseudokirchneriella subcapitata* demonstrated that the influence of cations was more complex than originally anticipated. Indeed, Ca^{2+} as well as Mn^{2+}, Cu^{2+} and Co^{2+} did not protect against Cd^{2+} toxicity over long-term Cd^{2+} exposure in this species, whereas Fe^{3+} and Zn^{2+} were effective protectors. We thus plan to study the effect of these base cations and essential micronutrients (within an environmentally realistic concentration range) on Cd^{2+} toxicity in a different green algal species (i.e. *Chlamydomonas reinhardtii*), in order to evaluate whether the effect of these essential cations on Cd^{2+} toxicity can be generalized to another similar organism. Uptake of Cd will be determined after short- and long-term exposures in the presence of several concentrations of Ca^{2+} and essential micronutrients. This project will contribute to the refinement of metal uptake/toxicity models such as the BLM and will lead to better predictions of metal-induced toxicity in freshwater algae growing under different chemical conditions.

Poster – Student Competition

Project Title:
(A5) Measured and modeled free copper in Canadian Shield lakes

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:
The bioavailability of metals varies as a function of their speciation and particularly the concentration of the free metal ion ([M^{z+}]). The binding of free metal ions by dissolved organic matter (DOM) influences metal speciation and the extent of this binding is thought to vary as a function of the "quality" of DOM. The WHAM chemical equilibrium model (WHAM = Windermere Humic Aqueous Model) takes into account free metal binding by DOM and is widely used within the Biotic Ligand Model construct to predict the bioavailability, and therefore toxicity, of metals to aquatic organisms in natural systems. Within WHAM, the user must choose what proportion of the measured DOC is "active", i.e. is involved in metal complexation. The ability to estimate this "percent active" spectroscopically would be of great interest. Our hypothesis is that the quality of DOM may be measured and incorporated into WHAM, to improve speciation predictions for natural aquatic systems.

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 Cu were determined by inductively coupled plasma-mass spectrometry (ICP-MS). The free metal concentrations (Cu^{2+}) were measured using a cupric ion-selective electrode and modeled using the WHAM v6.1 software. The concentration of DOM was also measured as dissolved organic carbon (DOC), whereas its quality was evaluated by both UV-visible absorbance and fluorescence. 3D-exciation/emission matrix (3DEEM) fluorescence spectra were analysed using the PARAFAC statistical analysis tool.

For 5 of the 18 lakes sampled, the measured Cu^{2+} concentration fell outside the envelope of values predicted by WHAM. PARAFAC analysis identified two fluorescence components contributing to the overall DOM fluorescence in the lakes sampled. Differences in the absolute fluorescence and the relative contribution of each fluorescence component were observed, both among lakes and between sampling regions. The relationship between the degree of metal complexation and the fluorescence quality of the DOM is being investigated.

Ultimately, the DOM fluorescence characteristics will be used to estimate the "active" DOM fraction, i.e. the fraction that participates in metal complexation reactions. This DOM fraction will be incorporated into WHAM in order to better predict the speciation of environmentally significant trace metals in aquatic systems.

Poster – Student Competition

Project Title:
(A5) Understanding Ni dynamics in the biomonitor *Chaoborus*

Investigator(s):
Ponton, Dominic and Hare, Landis
INRS-ETE, Université du Québec, Quebec City, Qc

Summary:
The phantom midge *Chaoborus* has been used to estimate bioavailable cadmium concentrations in lakewater. We have shown that this insect can also be used to monitor bioavailable nickel (Ni) concentrations, even in highly acidic lakes. In such lakes, H ions appear to outcompete Ni ions at biological uptake sites leading to little Ni bioaccumulation in spite of very high Ni concentrations in lakewater. Using a rational model, we were able to take into account this hypothesized competition between Ni and H ions and thereby improve the strength of the relationship between Ni in lakewater and in *Chaoborus* larvae. Furthermore, we were able to reproduce this competitive effect in the laboratory. To better understand the biodynamics of Ni in this insect we created a laboratory food chain using the green alga *Pseudokirchneriella subcapitata,* the cladoceran *Daphnia magna* and the phantom midge *Chaoborus flavicans*. The specific objectives of our study were to determine: (1) the relative importance of water and of food as Ni sources for both *Chaoborus* and *Daphnia*; (2) the efficiency with which *Chaoborus* assimilates Ni from its prey; (3) rates of Ni uptake from water by *Chaoborus,* as well as efflux rates of Ni from this insect. Our results show that Ni uptake by *Chaoborus* is mainly from water but that some Ni also enters via its food. The lack of importance of food as a Ni source is due in part to the fact that Ni assimilation from food is inefficient (it is also dependant on the predator's ingestion rate). Because water is the main Ni source, we measured Ni uptake from water by exposing *Chaoborus* larvae to Ni in water only 8 days and then measured Ni loss from this insect in uncontaminated water for an additional 8 days. We used all of these laboratory data to estimate model parameters such as the uptake rate of Ni from water (K_{uw}), Ni assimilation efficiency (AE) and the Ni efflux rate (K_e). Overall, our results suggest that there are substantial differences between Ni and Cd biodynamics in *Chaoborus* larvae. We suggest that *Chaoborus* larvae will be a useful component of risk assessment strategies designed to evaluate the Ni hazard for aquatic organisms in lakes.

Poster – Student Competition

Project Title:
(A5) Why bother to identify animals used for contaminant monitoring?

Investigator(s):
Proulx, Isabelle and Hare, Landis
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. Furthermore, they are a key step in using animals as contaminant biomonitors. Animal species used to achieve these ends are often pooled for contaminant analyses because it can be difficult, if not impossible, to separate them morphologically. However, by pooling species we assume that their contaminant concentrations do not differ markedly. We tested this assumption on the widespread midge *Chironomus* by collecting larvae of this genus from lakes located along a metal-contamination gradient and then identifying species using a combination of molecular and morphological techniques. By measuring trace metal concentrations in each *Chironomus* species we discovered that, within a given lake, the concentrations of some metals (e.g., Cu) did not differ among sympatric species, whereas those of other metals (e.g., Cd) did. Among lakes, metal concentrations in the various *Chironomus* species differed in a consistent manner such that certain species had consistently higher concentrations of some metals than did others. To determine why species sharing the same habitat should differ in their metal concentrations, we measured metal concentrations in larval gut contents, observed larval feeding behavior and measured larval sulfur isotopic ratios. These measurements revealed that *Chironomus* species sharing the same habitat tend to feed at different depths in sediment where metal bioavailability is likely to differ. Overall, our results suggest that a "one-size fits all" approach for *Chironomus* species may not be valid and that behavioral differences among these morphologically-similar larvae influence their exposure to contaminants. We suggest that considering species differences when using *Chironomus* and other aquatic animals to evaluate contaminant bioavailability, as contaminant biomonitors, or in laboratory toxicity tests could aid in producing more rigorous assessments of ecological risk.

Poster

Project Title:
(A8) The applicability of Biotic Ligand and Critical Residue Approaches to Canadian Shield conditions.

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

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:
To date the A8 research has focused is on studying two issues around the applicability of the biotic ligand approach in Canadian Shield conditions. The first project (MSc student Keegan Hicks) is directed at understanding the relative importance of natural organic matter (NOM) source on Cu bioavailability in soft water. The second project (MSc student Amanda Mancini) is trying to establish the applicability of biotic ligand models (BLMs) to fish species not traditionally used in toxicology. This latter project is also being developed in the context of the very soft waters typical of Shield conditions.

Amanda's project has compared the response to acute Cu or Cd exposure through 96 h LC50 tests and short term gill binding assays on rainbow trout, brook trout, lake trout, brown trout, lake whitefish and splake in soft water. Acute toxicity tests demonstrated that the response to Cu (reported last year) and the response to Cd differed among species. These results demonstrated that BLM based acute toxicity predictions must be adjusted to account for sensitivity differences among species. Short term gill accumulation experiments with Cu showed inconsistent patterns among species. For example brown trout had an elevated uptake capacity for Cu compared to other species and this was correlated to their higher sensitivity in acute toxicity tests. However splake, which was also sensitive to Cu did not have a higher uptake capacity. Therefore there was no clear link between short term accumulation and toxic impact (one of the theoretical assumptions within the BLM approach). Binding experiments designed to test the effects of cationic competition illustrated that all fish generally responded in a similar manner that was consistent with BLM principles (e.g. Na reduced Cu uptake while Ca and K did not). Results of binding tests with Cd showed very similar uptake among species (except for lake whitefish), demonstrating that toxicity differences were related to differences in sensitivity to the accumulated Cd rather than differences in accumulation.

Keegan's project, which includes work in the CANMET-MMSL labs in Ottawa with B. Vigneault, has involved 2 rounds of NOM sampling from softwater lakes (central Ontario and northern Quebec). His results clear show that the protective effect of NOM on Cu toxicity varies with source. Optical and chemical characterization techniques have also demonstrated clear differences between NOMs. The results from ion selective electrode measurements to assess Cu complexation characteristics showed differences in both the affinity and the maximum binding capacity with the latter being positively correlated with the protective effect on acute toxicity. In other words a higher capacity to complex Cu was

associated with a higher LC50. Excitation-emission matrix spectroscopy also showed differences between NOM sources however in this case there was no correlation with toxicity mitigation capacity. Specific absorption coefficient data confirmed a link between absorbance at 340 nm and reduced Cu uptake however some NOM sources with similar SAC340 values had very different toxicity mitigation capacities. Overall the results indicate that Cu complexation by NOM varies depending on source however the mechanism(s) by which this variability could be accounted for within the BLM is unclear.

Poster

Project Title:
(A8) Effect of environmental ligands on toxicity of nickel to Lemna minor

Investigator(s):
Gopalapillai, Y.;[1] Vigneault, B.;[2] and B.Hale[1]
[1]University of Guelph, Guelph, ON; [2]Natural Resources Canada, Ottawa, ON.

Summary:
The Biotic Ligand Model (BLM) approach, now incorporated in the regulatory framework for copper by the US EPA, is well-developed for predicting toxicity in aquatic fish and has been extended to invertebrate and algae. However, little information is available for BLM's application to aquatic plants such as the *Lemna minor*. *L. minor* is used to monitor mine effluent quality under the Metal Mining Effluent Regulations (MMER) and is sensitive to trace metals commonly discharged in mine effluents such as nickel. Prediction of Ni toxicity in *L. minor* was previously attempted using cross-species extrapolation of existing nickel BLMs and the results showed that the nickel toxicity data for *L. minor* was better explained by *Daphnia magna* BLM rather than the algae BLM. The effects of environmental ligands such as dissolved organic carbon (DOC) and flotation ligands on nickel toxicity to *L. minor* were studied to develop a bioavailability model for mine effluent and receiving waters. DOC's effect on reducing Ni toxicity was relatively small (less than a factor of 2) and can be explained by complexation alone. Alternatively, the ameliorating effect of flotation ligands was much less than expected based on estimated complexation. The long-term goal of this project is to develop a bioavailability model for *L. minor* which can be used for interpretation of the MMER monitoring data and for investigation of cause.

Poster – Student Competition

Project Title:
(A8) Cu Complexation by Natural Organic Matter: Linking Chemical Properties with Biological Effects.

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

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 effects of natural organic matter (NOM) source on Cu complexation were characterized both chemically and biologically in soft waters. NOM from different Canadian Shield soft water lakes were concentrated using reverse osmosis and for comparison NOM was also sampled from a hard water source. NOM complexation capacity was assessed directly in Cu spiked solutions (with and without additions of NOM) by measuring free Cu activity using an ion selective electrode. Additional chemical characterizations of NOM included specific absorbance coefficient (SAC; at 340nm), excitation-emission matrix spectroscopy, protein content, and molecular weight fractionation (fraction of DOC<1 kDa). The biological characterization of NOMs ability to complex Cu was measured in rainbow trout (*Oncorhynchus mykiss*) using short term (3h) gill accumulation, 96h-LC50, and the inhibition of gill Na/K ATPase. All NOM sources reduced Cu toxicity and 3h gill Cu accumulation. The protective effect of NOM on toxicity varied between sources where Allard River NOM was 3 fold more protective than Brandy Lake NOM. NOM source also varied in its ability to reduce gill Cu accumulation during 3h exposures to 2 µM Cu and either 2.5, 5 and 8 mg/L (DOC). The 3h gill accumulation data (at 2 µM Cu and 2.5 mg/L DOC) was negatively correlated with the 96h LC50s ($r^2 = 0.72$). The results from ion selective electrode measurements to assess Cu complexation characteristics of NOMs in solution showed differences in both maximum binding capacity (Bmax; 0.93 – 3.5 µmol Cu/mg C) and affinity (LogK; 5.97-6.45). Bmax values were positively correlated with the protective effect of NOM sources on acute toxicity ($r^2 = 0.57$) therefore suggesting that a higher capacity to complex Cu was associated with a higher LC50. Furthermore, the fraction of DOC less than 1 kDa was negatively correlated with toxicity ($r^2 = 0.80$) potentially suggesting that DOC with a larger molecular weight fraction may be more protective against Cu toxicity. These results on uptake and toxicity of Cu in soft waters were used to validate the biotic ligand model approach and with the goal to developing refinements in how NOM source differences can be incorporated.

Poster – Student Competition

Project Title:
(A8) Acute toxicity and gill accumulation of Cu or Cd in five species of fish in soft water.

Investigator(s):
Mancini, A., Hicks, K., and McGeer, J.
Department of Biology, Wilfrid Laurier University, Waterloo.

Summary:
The biotic ligand model (BLM) is currently the most advanced tool for predicting acute metal toxicity in aquatic systems based on site-specific water chemistry. The BLM has the potential for broad application, from water quality criteria/guidelines to risk assessment, however there are still some uncertainties associated with the approach. For example, BLMs for effects on fish are limited mostly to rainbow trout and fathead minnows in hard water and the applicability to other species and in soft waters is unknown. This project is concerned with testing the validity of Cu and Cd BLMs in very soft waters (representative of Canadian Shield conditions) using native fish species. The relative sensitivity different species was tested by comparing acute toxicity and gill accumulation responses to Cu and Cd. 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*) were tested under flow-through conditions in soft water (50 μM Ca, and conductivity of 30 μS). The 96 h LC50 values for Cu ranged from 3.5 μg/L for brown trout to >22 μg/L for lake trout. In tests with Cd the LC50 values ranged from 0.22 μg/L (for rainbow trout) to 1.53 μg/L (for brook trout), illustrating that sensitivity varied across species. Cu (or Cd) accumulation was measured in gills following short term (3-hr) exposures to various concentrations of dissolved metal and relationships between exposure and accumulation were used derive kinetic uptake variables. There were generally few differences among species except for a slightly higher Bmax in brown trout for Cu and in whitefish for Cd. In other short term exposure experiments, the effect of cationic competition on gill Cu accumulation in different species was studied. Fish were exposed to 9 μg/L Cu and varying concentrations of Na^{+}, Ca^{2+} or K^{+}. As expected from BLM, Cu accumulation is inhibited by increased levels of Na^{+}. The degree of protection provided by Na^{+} varied among species, but increased levels of Ca^{2+} in exposure medium had no effect on Cu binding. Overall the results indicate that the impacts of Cu and Cd vary across species and that differences in sensitivity are not directly related to metal uptake. Therefore within the BLM it is appropriate to account for differences among species in their response to Cu (or Cd) via adjustments of the sensitivity parameters in the model (as opposed to adjustment of uptake/accumulation parameters). Measured Cu accumulations were compared to BLM predictions of accumulation. The BLM underestimated both Cu accumulation (for the binding tests with increased Na), and toxicity (in terms of the LC50 values) and this is the topic of ongoing studies.

Poster

Project Title:
(A9) Is the Biotic Ligand Model an appropriate model for predicting bioaccumulation? Development of an analytical tool to evaluate Cd bioavailability in metal mixtures and freshwaters

Investigator(s):
England, R., Simon, D., and Wilkinson, K.J.

Summary:
This study is designed to evaluate the biological effects of bioaccumulation in metal mixtures and natural freshwaters. Peptides, including phytochelatins are known to be produced in response to metal exposures, allowing the organism to maintain homeostasis. This project was focused on the detection and quantification of the phytochelatins (PC2, PC3 and PC4) following their induction by Cd in the green alga, *Chlamydomonas reinhardtii* in freshwaters and simulated freshwaters. The first step of the project consisted in developing and optimizing a chromatographic method to separate and detect the different phytochelatins. For this, liquid phase chromatography coupled with tandem mass spectrometry using an electronebulizer with triple quadrupole analyser in selected reaction monitoring (SRM) mode was used. This technique should allow the detection of the analytes in the femtomole range. Subsequently, the extraction of phytochelatins from algal solutions was optimized for cadmium concentrations of 1.0×10^{-7} M, 5.0×10^{-7} M and 1.0×10^{-6} M. In the near future, field samples (already collected) and metal mixtures will be analyzed for their phytochelatin contents.