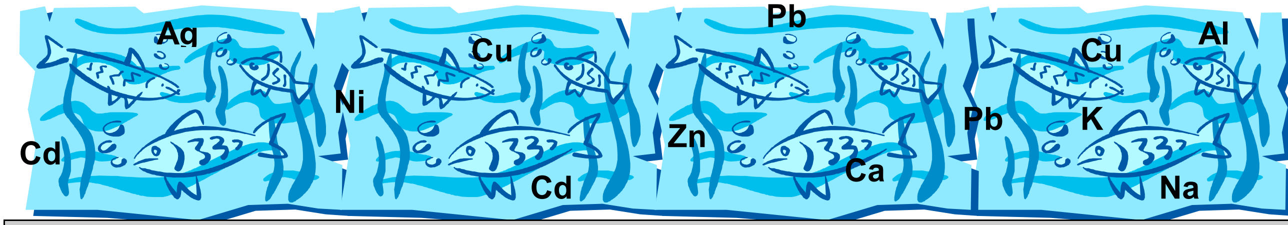
NSERC – Industry Project on Metal Bioavailability
Research Newsletter



Vol. 13: No. 1 ***McMaster University/Wilfrid Laurier University***
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News

Completion of NSERC CRD Grant Project (2005-2009)

The 4-year NSERC CRD grant project entitled "The science needed for site-specific regulation of metals in the aquatic environment – Improving and extending the biotic ligand model for ambient water quality criteria" was officially completed in August this year. We assessed the effects of 5 metals (Cu, Cd, Zn, Pb and Ni) that are considered of the greatest regulatory concern. This project involved diverse collaboration and active partnership between two universities (McMaster & Wilfrid Laurier University), a government laboratory (Natural Resources Canada - CANMET) and 7 international industrial research organizations (International Zinc Association/International Lead Zinc Research Organization, Copper Development Association/ International Copper Association; Vale Inco. Ltd., Golder Associates Ltd., Teck Cominco Metals Ltd., Xstrata Zinc Ltd., and the Nickel Producers Environmental Research Association). This project was productive. In total, we have produced 48 primary research papers in international peer-reviewed journals, 5 manuscripts in the process of publication or review; and 4 book chapters/ technical reports. In addition, we have participated in 90 conferences, seminar presentations and workshops. We are extremely grateful to all those involved, which included 6 Postdoctoral fellows, 5 Ph.D., 7 M.Sc., 12 undergraduate students, 4 technicians and 1 visiting scientist. The collective effort of the research team and the industrial partners has made this work a success and a great contribution to applying the BLM for site-specific risk assessments and regulation in Canada and abroad.

Congratulations

We are pleased to announce that two students who were actively involved in the NSERC CRD grant project have graduated this year. Paul Craig, who was supervised by Grant McClelland and co-supervised by Chris Wood, successfully defended his Ph.D. thesis in July. Paul contributed by developing genomic endpoints for acute and chronic toxicity of Cu in zebrafish. He now holds a postdoctoral fellowship at University of Ottawa. Matthew Clifford, who was a M.Sc. student with Jim McGeer at Wilfrid Laurier University, also defended his M.Sc. thesis in the same month. Matt was involved in developing a BLM for Cd and Zn in *Daphnia magna* in softwater environments. He also examined the waterborne and dietborne toxicity of Cd to *Hydra*. Matt has moved on to start his studies in medical school this autumn.

Nish Pais who did her 4th year undergraduate thesis project with Chris Wood, mentored by Dr. Tania Ng, also graduated in April this year. She was involved in the first year of NSERC Strategic grant project (2008 – 2010) by examining the acute toxicity and bioaccumulation of Cu in three freshwater invertebrates – *Lymnaea stagnalis, Lumbriculus variegatus* and *Chironomus riparius* in hardwater. She has recently started her M.Sc. study with Jim McGeer in Wilfrid Laurier University. She is still partly involved in the Strategic project, working on toxicity and accumulation of Cd and Cd nanoparticles (quantum dots) in *L. stagnalis*,

Welcome

This year, we have four new 4th year undergraduate students who are involved in metal research in our lab. Josh D'Silva mentored by Ph.D. student Erin Leonard, will work on chronic toxicity and bioaccumulation of Ni in rainbow trout. Tarunpreet Dhaliwal, mentored by Dr. Tania Ng will examine Cu toxicity and bioaccumulation in chironomids and *L. variegatus* using both single and combined exposure regimes. Margaret Tellis, mentored by Dr. Derek Alsop, will investigate the effect of Cu on cortisol release in rainbow trout. Anu Singh, mentored by Joel Klinck, will examine the mechanism of Cd and Ca uptake in gastrointestinal tract of rainbow trout. Both Josh and Tarun's research are supported by the Strategic Grant.

Dr. Scott Smith started his sabbatical research in Chris Wood's lab since September. Scott is an Associate Professor of Chemistry at Wilfrid Laurier University and his research interest is on environmental chemistry of metals in aquatic environments, and particularly their interactions with DOC. His current research focuses on the interactions of metals with biological surfaces.

Dr. Ishaq Ahmed, a postdoctoral fellow under the supervision of Dr. Greg Pyle at Lakehead University in Thunder Bay, visited McMaster for metal research on fathead minnows several times this year. His research is supported by supplementary funding from the Metals in the Human Environment Strategic Network (MITHE – SN) to the primary investigators, Greg Pyle (Lakehead University), Chris Wood (McMaster University) and Dr. Patrice Couture and Dr. Peter Campbell (INRS, University of Quebec). This research examines the effects of waterborne and dietborne Cu on genomic responses of gills, gut, muscle, and olfactory epithelia as well as on physiology, neurophysiology, behaviour, gene expression and enzymatic responses of fathead minnows. Ishaq, with the assistance of Sunita Nadella measured the Cu uptake via gill and gut of fathead minnows after they were exposed to waterborne and dietborne Cu earlier this year. He re-visited McMaster recently to quantify gene expressions by realtime PCR, in order validate microarray results and identify important functional genes in the tissue of these Cu-exposed fish.

Camilla Martins, a Ph.D. student under the supervision of Dr. Adalto Bianchini, at the University of Rio Grande in southern Brazil, spent 5 months visiting the Wood lab this past summer where she examined the mechanisms of Cu uptake in the estuarine blue crab. Camilla is the first of many senior graduate students who will be exchanged between the two labs in Canada and Brazil over the next 5 years under the new International Research Chair Program awarded to Adalto Bianchini and Chris Wood last spring (see below).

Welcome to the new people who join and visit our lab! We are excited for any insights from their research collaboration.

New funding and equipment

This past spring, Drs. Adalto Bianchini and Chris Wood were very pleased to receive a 5-year funding award from IDRC, The International Development and Research Council of Canada. The award supports an International Research Chair for Adalto at the University of Rio Grande and an associated research program in Coastal Zone Management in Brazil. This program is coupled to Chris' Canada Research Chair in Environment and Health at McMaster University. Metals, social change, integrated management strategies, and graduate student training are some of the key themes of the program. Environment Canada (Dr. Patty Gillis), the International Copper Association (Dr. Bob Dwyer), the United Nations University-Water (Dr. Adeel Zafar), and INPA (the Amazon Research Institute of Brazil; Dr. Adalberto Val) are some of the key partners. The program begins officially in November; Chris will be traveling to Brazil at this time for the inaugural workshop. Ph.D. student Erin Leonard will be the first Canadian postgraduate traveling to Brazil in the spring of 2010 and 3 Brazilian doctoral students will visit McMaster at that time for periods of 4-12 months.

Chris Wood and Grant McClelland (McMaster University) and Jim McGeer, Scott Smith, and Mike Wilkie (all at Wilfrid Laurier University) were pleased to receive equipment funding from an NSERC RTI application this year. A new Shimadzu TOC-Vcph Total Organic Carbon Analyzer has been purchased and set up in the Wood lab. Installation and training have been completed. It will be mainly used for measuring dissolved organic carbon in metal research.

Chris Wood was also pleased to receive funding from the International Zinc Association and from the International Lead Zinc Research Organization to study the effects of lead and zinc at varying dissolved organic carbon and salinity on the development of mussel embryos, as well as the early life stages of other marine invertebrates. Chris Wood, Sunita Nadella, and summer technician Jaquie Beaudry went to the Bamfield Marine Science Centre, Vancouver Island this past summer to complete the first round of experiments aimed at generation of data for marine and estuarine BLM's. Adalto Bianchini and Scott Smith are also contributing their expertise to this project.

Jim McGeer was awarded an NSERC RTI this spring for the purchase of a swim flume. The equipment will be used to assess swim performance during sublethal exposure.

Conference presentations

The following papers were presented by the Metals Bioavailability Group in the 48th Annual Meeting of the Canadian Society of Zoologists, Toronto, Ontario. May 12–16, 2009.

- **Alsop, D., Wood, C. M. (2009)**. Development of a high-throughput toxicity assay with zebrafish larvae: testing metal mixtures.
- **Ng, T. Y.-T., Chowdhury, J. M., Wood, C. M. (2009)**. Does the biotic ligand model (BLM) predict the influence of pH on Cu toxicity to the softwater-acclimated rainbow trout?
- **Milne, J. L., McGeer, J. (2009).** Dynamics of chronically accumulated cadmium in trout.
- **Clifford, M., Costa, E. J., McGeer, J. (2009).** Effects of waterborne and/or dietary cadmium on *Hydra attenuata*.
- **Straus, A., McGeer J. (2009)**. Cd bioaccumulation in *Lumbriculus variegatus* and *Chironomus riparius*.

The following papers will be presented by the Metals Bioavailability Group at the SETAC North America 30th Annual Meeting, New Orleans, Louisiana, USA. Nov 19-23, 2009.

- **Al-Reasi, H., Wood, C. M., Scott, S. (2009).** Characterization of natural organic matter (NOM): Implications for metal toxicity in aquatic environments.

- **Costa, E.-J., Sadlier, L., Clifford, M., McGeer, J. (2009).** Influence of water chemistry on the sublethal effects of Cu to *Hydra attenuata*.

- **Cunningham, J., Parkinson, K., McGeer, J. (2009).** The effect of chronic Cd exposure on swim performance in rainbow, brook and brown trout.

- **Klinck, J. S., Wood, C. M. (2009)**. A comparison of calcium and cadmium uptake along the gastrointestinal tract between freshwater and seawater acclimated *Oncorhynchus mykiss*.

- **Leonard E. M., Wood, C. M. (2009).** Development of a tissue residue approach for the risk assessment of Ni in the Canadian freshwater environment.

- **Mancini, A., Milne, J., McGeer, J. (2009).** Acute toxicity and chronically-induced physiological effects during waterborne Cd exposure for numerous fish species.

- **Milne, J., Mancini, A., McGeer, J. (2009).** Tissue and subcellular distribution of chronically accumulated Cd in rainbow trout (*Oncorhynchus mykiss*).

- **Nadella, S. R., Ahmed, I. V. P., Pyle, G., Wood, C. M. (2009).** Copper uptake kinetics in the gills and gut of fathead minnows exposed to waterborne vs dietary copper.

- **Ng, T. Y.-T., Chowdhury, J. M., Wood, C. M. (2009)**. Does the biotic ligand model (BLM) predict pH effects on Cu toxicity to the softwater-acclimated rainbow trout?.

- **Ng, T. Y.-T., Pais, N., Wood, C. M. (2009)**. Development of a tissue residue approach for risk assessment of Cu using *Lymnaea stagnalis*, *Lumbriculus variegatus* and *Chironomus riparius*.

- **Scott, S. Al-Reasi, H., Gheorghiu, C., DePalma, S., Hicks, K., McGeer, J., Playle, R., Wood, C. M. (2009).** Molecular spectroscopy to differentiate dissolved organic matter source and implications for metal speciation and toxicity.

- **Wood, C. M., Grosell, M., McDonald, D., Walker, P., Playle, R. C., Walsh, P. (2009).** Chronic exposure to waterborne silver in a marine teleost, the gulf toadfish (*Opsanus beta*): Effects of feeding on bioaccumulation and physiological responses.

The following papers were presented by the Metals Bioavailability Group at the Aquatic Toxicity Workshop 36th Annual Aquatic Toxicity Workshop, La Malbaie, Quebec, Sept 27-30, 2009.

- **McGeer, J., Costa, E. J., Clifford, M., Sadlier, L. (2009).** Dietary and waterborne toxicity of Cd and Cu to *Hydra Attenuata*.

- **Khan, F., Keller, W., Gunn, J., Welsh, P., Yan, N., Wood, C. M., McGeer, J. (2009).** Application of metal bioavailability and impact models to predict biological recovery of metal contaminated lakes in the Sudbury area.

The following peer reviewed papers were published by the Metals Bioavailability Group in November 2008 - October 2009

- **Craig, P. M., Galus, M., Wood, C. M., McClelland, G. B. (2009).** Dietary iron alters waterborne-copper induced gene expression in softwater acclimated zebrafish (*Danio rerio*). Am. J. Physiol. R. 296: R362-373.

- **Klinck, J. S., Ng, T.Y.-T., Wood, C. M. (2009).** Cadmium accumulation and in vitro analysis of calcium and cadmium transport functions in the gastro-intestinal tract of trout following chronic dietary cadmium and calcium feeding. Comp. Biochem. Physiol. C. 150:349-360.

- **Kozlova, T., Wood, C. M., McGeer, J. C. (2009).** The effect of water chemistry on the acute toxicity of nickel to the cladoceran *Daphnia pulex* and the development of a biotic ligand model. Aquat. Toxicol. 91:221-228.

- **Leonard, E. M., Nadella, S. R., Bucking, C., Wood, C. M. (2009).** Characterization of dietary Ni uptake in the rainbow trout, Oncorhychus mykiss. Aquat. Toxicol. 93:205-216.

- **Bechard, K., Gillis, P.L. and Wood, C.M. (2008).** Trophic transfer of larval chironomids (*Chironomous riparius*) exposed via sediment or waterborne routes, to zebrafish (*Danio rerio*): tissue specific and subcellular comparisons. Aquat. Toxicol. 89:310-321.

- **Birceanu, O., McGeer, J.C., Chowdury, M.J., Gillis, P., Wood, C.M. and Wilkie, M. (2008)**. Modes of metal toxicity and impaired branchial ionoregulation in rainbow trout exposed to mixtures of Pb and Cd in soft water. Aquat. Toxicol. 89:221-231.

- **Clifford, M. and McGeer, J. C. (2008).** Influence of water chemistry on the toxicity of Zn to *Daphnia pulex* in soft waters. Aquat. Toxicol. 91:26-32.

- **Galvez, F., Donini, A., Smith, S., O'Donnell, M., and Wood, C.M. (2008).** A matter of potential concern: Natural organic matter alters the electrical properties of fish gills. Env. Sci. Tech. 42: 9385-9390.

- **Nadella, S., Fitzpatrick, J.L, Franklin, N., Bucking, C.P., Smith, S., and Wood, C.M. (2008)**. Toxicity of dissolved Cu, Zn, Ni and Cd to developing embryos of the blue mussel (*Mytilus trossolus*) and the protective effect of dissolved organic carbon. Comp. Biochem. Physiol. C. 149: 340-348.

- **Ng, T., Klinck, J. and Wood, C.M. (2008)**. Does dietary Ca protect against toxicity of a low dietborne Cd exposure to the rainbow trout? Aquat. Toxicol. 91:75-86.

- **Niyogi, S., Kent, R., Wood, C.M. (2008).** Effects of water chemistry variables on gill binding and acute toxicity of cadmium in rainbow trout (*Oncorhynchus mykiss*): a biotic ligand model (BLM) approach. Comp. Biochem. Physiol. C. 149:305-314.

- **Ojo A.A. and Wood C.M. (2008).** *In vitro* examination of interactions between copper and zinc uptake via the gastro-intestinal tract of the rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. 56:244-252.

The following peer reviewed paper by the Metals Bioavailability Group is in press:

- **Craig, P. M., Hogstrand, C., Wood, C. M., McClelland, G. B. (2009).** Gene expression endpoints following chronic waterborne copper exposure in a genomic model organism, the zebrafish, *Danio rerio*. Physiol. Genomics. In Press.

Research Highlights

This issue will highlight research conducted by Matthew Clifford (M.Sc. student) at Wilfrid Laurier University under the supervision of Jim McGeer. Matt's work is currently working its way through the publication process.

Waterborne and dietary effects of cadmium on *Hydra attenuate*

Matthew Clifford and Jim McGeer
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The biotic ligand model (BLM) has recently been developed to predict the interaction between dissolved metals and toxic effects in aquatic organisms (Di Toro et al., 2001). The strength of this geochemical equilibrium approach and the robustness of the physiological underpinning have led to the application of this toxicity prediction model for water-quality guideline and criteria derivation (EPA, 2007). The BLM does not account for potential effects that occur via dietary exposure routes. In fresh waters, the site of metal uptake during waterborne exposures is primarily at the respiratory epithelium (gills in fish) and effects have been well documented for a number of metals. Incorporation of dissolved metal into food represents a potentially important route of exposure for prey organisms but less is known about dietary uptake and the potential for associated impacts (reviewed Handy et al., 2005).

Hydra (Cnidaria: Hydrazoa) are freshwater organisms commonly found in slow moving rivers and streams (Beach and Pascoe 1998). Hydra have been used to assess the toxicity of a variety of contaminants in freshwater (Hyne et al., 1992; Pollino and Holdway, 1999; Holdway et al. 2001; Quinn et al., 2008). With the exception of the pioneering work of Karntanut and Pascoe (2007), who studied the bioaccumulation of Cu, Cd, and Zn in *Hydra vulgaris* from contaminated *Artemia nauplii*, the effects of metal-contaminated food on Hydra do not appear to have been studied.

This study was divided into two major parts. (I) We investigated the effects of water geochemistry on waterborne Cd toxicity by manipulations of water ions (e.g. Ca, Mg, Na, K, and Cl), pH and dissolved organic matter (DOM) with a view to developing a BLM for Hydra. (II) We assessed the relative toxicity of waterborne and dietary Cd to *Hydra attenuata*. *Daphnia pulex* were used as the source of dietary Cd to Hydra.

Part I

H. attenuata responded to waterborne Cd in a dose-dependent manner for both lethal and sublethal endpoints with the latter being about 3 fold lower (data not shown). In the sub-lethal data set Ca was tested over a range of 0.2-1.21 mM and a 4 fold reduction in EC50 values, from 0.18 to 0.79 μM, was observed. In the lethal endpoint study Ca concentrations ranged from 0.13 to 1.31 mM and the LC50 values increased by approximately 3 fold (from 0.62 to 1.53 μM). The relationships for Ca^{2+} on sub-lethal Cd^{2+} EC50 free ion activity based toxicity is shown in Figure 1A.

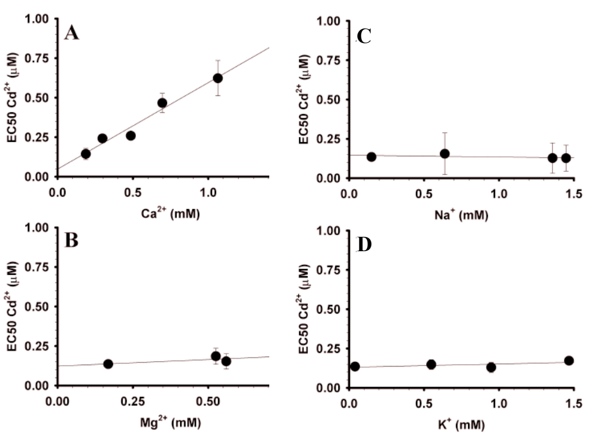

Figure 1. Measured EC50s (with 95% confidence intervals) for the effects of Ca^{2+} (panel A), Mg^{2+} (panel B), Na^{+} (panel C) and K^{+} (panel D) on sub-lethal Cd^{2+} toxicity to H. attenuata. Only slope of regression line for Ca effect is significantly different from zero.

The slopes of the effects of Ca^{2+} on sub-lethal and lethal Cd^{2+} endpoints were similar, indicating a consistent competitive effect. The intercept of the line of regression for the sub-lethal end point was 9 times lower than that of the lethal end point. These intercepts were used to estimate the Log K_{CdBL}, the binding of Cd to the biotic ligand in the absence of competitive interactions, yielding values of 6.4 for the lethal endpoint and 7.3 for the sublethal endpoint.

In sub-lethal and lethal data sets we found no toxicity mitigating influence from Mg, Na and K indicating no competitive interaction between these ions and Cd^{2+} in Hydra (see Figure 1B, C & D for sublethal).

The effect of NOM on Cd toxicity was assessed for the lethal endpoint and there was a small (approx 20% increase) but significant increase in LC50. These differences were not sufficient to produce a significant slope to the regression relationship between NOM concentration and total dissolved Cd. The effects of pH were not tested because Hydra proved to be very sensitive to pH changes.

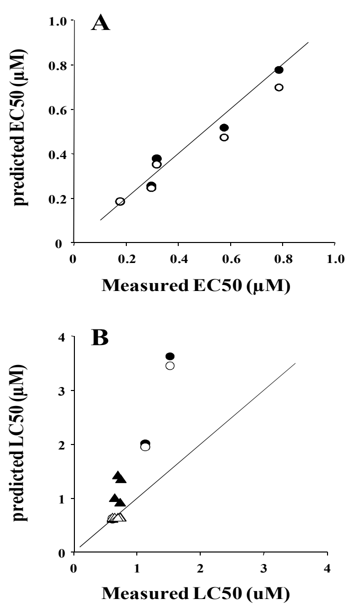

Figure 2. Ability of modelling approaches to predict the measured sublethal toxicity (EC50s, Panel A) and lethality (LC50 Panel B) of Cd to Hydra attenuata. In both panels the prediction estimates are given for the Ca test series using the BLM (filled circles) as well as the adjusted CMC hardness equation (open circles). For LC50s (Panel B) predictions for the NOM test series is also shown (closed triangles is BLM and open is adjusted CMC hardness equation). Both panels the solid line shows where predicted values are equal to measured.

The HydroQual BLM was used to develop predictions of Cd toxicity to Hydra. There is no Hydra-specific BLM and therefore the one developed for *Ceriodaphnia dubia* was used. Following adjustments of the LA50 value (the predicted accumulation associated with 50% effect, also known as the critical value) to 6.95 nmol Cd/g, a good match between measured and predicted EC50 was evident (Figure 2A). In the case of LC50s the LA50 value in the model was adjusted up to 7.74 nmol/g to give a good match between measured and predicted at low Ca levels (Figure 2B). The predicted toxicity at high Ca levels showed that the BLM over-estimated Cd LC50s (Figure 2B). The actual protective effect of NOM on acute lethality was also much less than predicted by the BLM (Figure 2B). The EPA CMC hardness equation was also tested for its ability to predict the protective effect of Ca. The CMC is designed for extremely sensitive organisms and it was necessary to multiply hardness adjusted CMC values by constants of 28 and 132 (EC50 and LC50 series respectively) to bring estimates up to a level where trends in Ca protection could be compared. In both cases the estimates provided by the adjusted CMC Cd hardness values were remarkably close to BLM prediction values (Figure 2).

Part II

In the first waterborne only test, the EC50 for tentacle regression in hard water (140 mg/L as $CaCO_3$) was 0.79 µM Cd in water with a hardness of 140 mg/L while in softer water it was significantly less at 0.30 µM. Daphnia were used as the source of dietary Cd and 24 h was determined as a time suitable for them to come to equilibrium during exposures. In spite of very different waterborne Cd exposure concentrations in solutions of 40 and 140 mg/L hardness, the accumulation of Cd was similar and accumulation data (Fig 3A and B) was fitted to an exponential model that gave estimates of the saturation concentrations.

Whether in hard water or in soft water, Hydra fed *Daphnia pulex* contaminated with Cd (at the Hydra EC50 concentration) showed no effects (Fig 4). As well, Hydra exposed to waterborne Cd plus dietborne Cd showed there was no additivity as no additional tentacle clubbing occurred beyond that induced by waterborne Cd alone. This unequivocally shows that the effects of waterborne Cd are more important than those arising from dietary exposures.

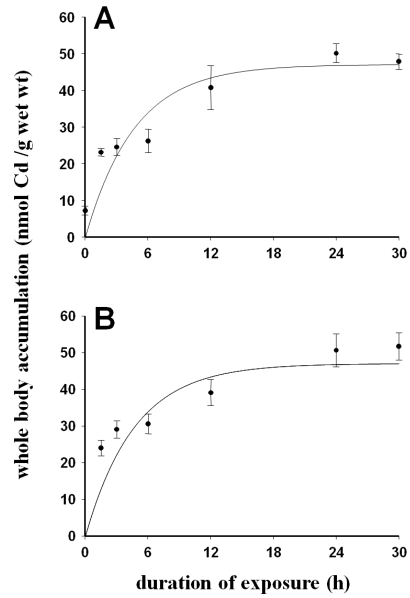

Figure 3. Whole body bioaccumulation of Cd in 7 to 8 day old Daphnia pulex (mean ± SEM) over 24 h of exposure to 0.31 µM Cd at a hardness of 40 mg $CaCO_3$/L (Panel A) or 1.02 µM Cd at a hardness of 140 mg $CaCO_3$/L (Panel B). Best fit lines using an exponential model are given with r^2=0.90 of Panel A and 0.87 for Panel B, see text for other model parameter details. Each mean represents n=15 Daphnia at low hardness (A) and n=13 for high hardness (B).

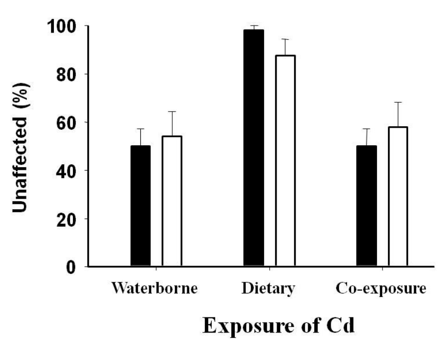

Figure 4. Percentage of Hydra displaying no clubbed tentacles (i.e. unaffected Hydra) as a result of the waterborne, dietborne, or combined water plus dietborne Cd to H. attenuata following a 96 h exposure. Bars show mean ± SEM percent of unaffected Hydra at exposures of either 0.41 µM Cd in solutions at a hardness of 40 mg $CaCO_3$/L (dark bars, n=48) or 1.42 µM Cd in solutions at a hardness of 140 mg $CaCO_3$/L (light bars, n=24). For dietary exposure Daphnia pulex were exposed to these same waterborne exposure concentrations for 24 h prior to feeding to Hydra.

During waterborne exposure the majority of cells in Hydra (external epidermal as well as gastrodermal) are exposed to Cd. During dietary exposure only the gastrodermal cells are exposed. Little is known about Cd uptake in Hydra but it may be that the uptake and impacts of Cd differs across cell types. It is also possible that the accumulated Cd present in the *Daphnia* was sequestered by metallothioneins (MT) or other metal binding like-proteins. Work by Fraysse et al., (2006) has shown that as much as 57% of the total accumulated Cd is bound to MTs in *Daphnia magna.* Daphnia have been shown to increase synthesis of these proteins in as little as 2-24 h (Amiard et al., 2006) and therefore it is also possible that the Cd accumulated by the Daphnia was bound to the MT and not available for uptake. It is also important to highlight that Hydra do not consume all of the Daphnia as undigested particles (e.g. the carapace) are eliminated from the gut space.

Acknowledgements: The funding for this project was through a NSERC Strategic Grant with additional support from Rio Tinto Alcan and including in-kind participation from the Environmental Effects Monitoring Program of Environment Canada. The NSERC CRD program in collaboration with ICA, CDA, NiPERA, ILZRO, IZA, Teck Resources, Xstrata Zinc, Vale Inco, and Golder Associates (Dr. Peter Chapman) also made key contributions.

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