Aquatic Toxicology 91 (2009) 221–228

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

The effect of water chemistry on the acute toxicity of nickel to the cladoceran *Daphnia pulex* and the development of a biotic ligand model

Tatiana Kozlova[a,c], Chris M. Wood[a], James C. McGeer[b,c,*]

[a] Department of Biology, McMaster University, Hamilton, ON, Canada
[b] Department of Biology, Wilfrid Laurier University, Waterloo, ON, Canada
[c] CANMET-MMSL, Natural Resources Canada, Ottawa, ON, Canada

ARTICLE INFO

Article history:
Received 17 July 2008
Received in revised form 30 October 2008
Accepted 5 November 2008

Keywords:
BLM
Ni
WHAM
Modeling
Cladocerans
Water hardness
Bioavailability
Metal risk assessment

ABSTRACT

The goal of this study was to evaluate the influence of water chemistry parameters on the acute toxicity of waterborne Ni to *Daphnia pulex* in soft waters and using this information to develop a biotic ligand model. The effects of Ca, Mg, Na, K, Cl, pH (two differently buffered sets) and natural organic matter (NOM) from two sources were evaluated in standardized 48 h acute toxicity tests. Increases in Ca^{2+} had a protective effect on Ni toxicity, suggesting that this ion competes with Ni at the site of biological uptake. Increased waterborne Mg^{2+} also reduced Ni toxicity, but to a lesser degree compared with Ca^{2+}. EC50 values increased at higher pH when the organic buffer 3-morpholinepropanesulfonic acid was used to adjust test pH, however in tests series where pH was varied using HCO_3^- the results were equivocal. Other testing showed that Na, K and Cl did not influence the toxicity response of *D. pulex* to Ni. Complexation of Ni by NOM reduced toxicity but Nordic Reservoir NOM was much more protective compared to Suwannee River NOM. Geochemical modeling of organic matter complexation of Ni was done using the HydroQual Biotic Ligand Model (BLM ver. 2.3.3; research mode) and the Windermere Humic Aqueous Model (WHAM ver 6.0). Results showed dramatic differences between the two models in dissolved organic matter complexation. Modelling of Ni geochemistry for test solutions other than those containing NOM showed consistent and minor differences between the WHAM and the BLM. The latter model was used to develop a comprehensive prediction model of Ni toxicity. log *K* values developed for competitive cationic effects showed that Ca and Mg have a much higher protective effect in soft water compared to models developed for *Daphnia magna* in hard water. The BLM developed for this species in soft water provided good predictions of toxicity across a wide range of Ni concentrations but also highlighted the need for an improved understanding of the effects of NOM and pH on Ni toxicity in soft waters.

1. Introduction

The acute toxicity of metals in aquatic systems is known to vary widely, dependent on both the sensitivity of the organism and environmental conditions. Water chemistry influences the speciation of metals and an understanding of the bioavailability and toxicity of different geochemical forms (species) have led to the development of comprehensive toxicity prediction models (Meyer et al., 1999; Di Toro et al., 2001). These biotic ligand models (BLMs) use the geochemical equilibrium modeling approach to provide site specific toxicity estimates by accounting for the chemical speciation of both the metal and other moieties in aquatic systems, bioavailability of the metal, and its physiological mechanism(s) of toxic impact (Paquin et al., 2002; Di Toro et al., 2001; Santore et al., 2001; De Schamphelaere and Janssen, 2002, 2004a,b; Keithly et al., 2004).

BLMs have been published for some metals (reviewed Niyogi and Wood, 2004; McGeer, in press) but the development of Ni BLMs is less advanced because the mechanisms of Ni toxicity and the influence of water chemistry on toxic responses are not well understood. Ni is an essential element in some organisms and plays a physiological role in lipid metabolism, hematopoiesis and other functions (Phipps et al., 2002; Anke et al., 1995; Nielsen, 1996). Naturally occurring concentrations of Ni in surface waters are generally in the low μg/L (e.g., between 0.5 and 5 μg/L) but can sometimes range as higher as 0.2 mg/L in catchments influenced by fine-grained sulphide bearing sediments (CCME, 1987; Astrom and Bjorklund, 1996; Zwolsman and van Bokhoven, 2007). Essentiality has been reported for terrestrial organisms but this is not well established in aquatic organisms (Muyssen et al., 2004; Chowdhury et al., 2008). Like other metals, Ni induces toxic responses when

* Corresponding author at: Department of Biology, Wilfrid Laurier University, 75 University Ave. West, Waterloo, ON, Canada N2L 3C5. Tel.: +1 519 884 0710 fax: +1 519 746 0677.
E-mail address: jmcgeer@wlu.ca (J.C. McGeer).

0166-445X/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.aquatox.2008.11.005

concentrations are elevated. The study of Pane et al. (2003a) linked the impacts of Ni on *Daphnia magna* in hard water to disruption of Mg balance in the organism. However, studies with rainbow trout showed that the acute impact of Ni was primarily as a respiratory toxicant (Pane et al., 2003b, 2004b).

BLM approaches for Ni have been developed and published for fish (Meyer et al., 1999; Hoang et al., 2004; Deleebeeck et al., 2007a). However, there are very few studies applying the BLM approach to the impacts of Ni on aquatic invertebrates (Keithly et al., 2004; Pane et al., 2003a) and most of these studies were done using hard or moderately hard waters. A BLM for the effects of Ni on *D. magna* has recently been published (Deleebeeck et al., 2007b) and it showed that water hardness cations (i.e., Ca and Mg) are protective. The inverse relationship between water Ca (and/or Mg) concentration and Ni toxicity makes the development of BLMs in soft water particularly relevant because many of the waters on the Canadian and Fennoscandian Shields have very low levels of calcium carbonate (Jeziorski and Yan, 2006). In a study of Canadian Shield lakes from the south and central region of Ontario, Ca concentrations ranged from 30 to 1070 μM (mean 94.3, $n = 100$) with 5% of the samples below 40 μM and 95% less than 200 μM (David et al., 1997). In these very soft waters Ni bioavailability and toxicity are expected to be elevated.

In this study, the effects of metal-toxicity modifying factors on Ni toxicity to *D. pulex* in soft water were investigated in order to contribute towards the development of an acute Ni BLM. The potential for Ca, Mg, Na, Cl, K, pH and natural organic matter (NOM) to alter the toxicity of Ni to *Daphnia pulex* was tested. *D. pulex* was chosen as the test organism because of its sensitivity to metals (Shaw et al., 2006) as well as its ability to acclimate and thrive in very soft waters (Environment Canada, 1996). Clifford and McGeer (2009) have recently developed a BLM for Zn in soft water for this same species.

2. Materials and methods

2.1. D. pulex culturing

D. pulex were obtained from a commercial supplier (Aquatic Research Organisms, Hampton, NH) and cultured at 21 °C in 1L beakers with approximately 800 mL of reconstituted soft water (see below for composition). A mixture of two algae species, *Chlorella vulgaris* (30%) and *Pseudokirchneriella subcapitata* (70%), originally obtained from the University of Toronto Culture Collection, was fed to the *Daphnia* cultures and this was supplemented with a yeast, cerophyll and trout feed suspension (YCT, purchased from Aquatic Research Organisms). Algae feed preparation and *D. pulex* feeding were carried out according to standard methods (Environment Canada, 1996) and a vitamin pre-mix containing B_1, B_7, and B_{12} in equal proportion was added to the culture water at a total concentration of 0.1 mg/L. The culture and testing of *Daphnia* were carried out at 20.5 ± 1 °C and photoperiod was fixed at 16 h of light and 8 h of dark; culture beakers were not aerated. Neonate daphnids less than 24 h of age were used for testing and the first brood of neonates was not used (Environment Canada, 1996).

Culture and testing were done at the Mining and Mineral Science Laboratories of Natural Resources Canada in Ottawa. A reconstituted soft water medium, created by the addition of $CaSO_4$, $MgSO_4$, $NaHCO_3$, and KCl (purchased from Sigma–Aldrich, ON, Canada) to deionized water, was used for culture and as the base for test solutions. The composition of the standardized soft water medium recommended by Environment Canada (1996) and the USEPA (2002) was adjusted with a reduction in Mg content to give final nominal concentrations of: 170, 140, 570, 30, 30, 310 and 570 μM for Ca, Mg, Na, Cl, K, SO_4^- and HCO_3^- respectively. The reduction of Mg was done to bring the Ca:Mg ratio closer to that found in natural waters (David et al., 1997). *D. pulex* cultures were acclimated for at least 30 d prior to testing, water was fully aerated before use, the pH of the medium was 7.8, and the dissolved organic carbon (DOC) content was measured at 0.15 mg/L.

2.2. Toxicity testing

Static acute 48-h assays were conducted according to a standard Biological Test Method from Environment Canada for *Daphnia* (Environment Canada, 1996). Immobility at 48 h was the bioassay endpoint, assumed to equivalent to mortality. For each test a control treatment and at least 7 Ni exposure concentrations were tested using two replicates for each concentration with 10 neonates per replicate. Within each test Ni exposure concentrations were Ni was added to test solutions as $NiSO_4$ and in preliminary testing potential differences between $NiSO_4$ and $NiCl_2$ were compared. There were a total of 44 toxicity tests within 8 test series. A test series consisted of holding all water variables constant except for one, which was varied to different levels in each toxicity test. Test series are outlined in Table 1 and included Ca (as $CaSO_4$), Mg (as $MgSO_4$), Na (as NaCl) and K (as KCl). Two different series were done adjusting pH. In one $NaHCO_3$ (pH, alkalinity and Na varied) was used and in the other the organic buffer 3-morpholinepropanesulfonic acid (MOPS, purchased from Fisher Scientific Inc.) at 750 mg/L (De Schamphelaere et al., 2004) was used to control pH in conjunction with small volumes of either 1 N HCl or NaOH as appropriate. These series were designated as pH set 1 and 2 respectively (see Table 1). Test solutions were left to equilibrate for 24 h before initiating tests. Two natural organic matter (NOM) sources were tested, Suwannee River NOM (SRNOM) and Nordic Reservoir NOM (NRNOM). In these tests additions of NOM were added directly to culture water to achieve a variety of different DOC concentrations (Table 1). NOMs were obtained from the International Humic Substances Society (IHSS, St. Paul, MN).

2.3. Sampling and characterization

The pH, conductivity, dissolved oxygen and temperature of test media were measured prior to initiating a test as well as after 48 h. Water samples (10 mL) were collected at 0 and 48 h for Ni and element analysis and these were acidified to 1% using 16 N HNO_3. Samples for DOC measurement were not acidified, but were stored at 4 °C. All samples were filtered (0.45 μm Acrodisc HT Tuffryn Membrane, PALL, NY). To confirm whether the added Ni in solution was all in the dissolved phase, unfiltered samples were additionally collected in a few of the test series and total Ni concentration measured.

The pH of solutions was measured using a PHM290 Meter with pHC2701 electrode (Radiometer, Copenhagen). Conductivity was measured using a conductivity meter (Orion model 1230, ORION Research, Inc., Beverly, MA) and dissolved oxygen levels were assessed using an IonCheck 20 meter (Radiometer). Measurements of Ni, Ca, Mg, Na, K, and Cl in water samples were done via inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian Inc.). The limit of quantification for Ni by this method was 0.15 μM (9 μg/L). DOC was measured using a total organic carbon analyzer (TOC-VCPH/CPN, Shimadzu). Alkalinity was measured using an automated titrator (Metrohm 751 GPD Titrino, Westbury, NY). Hardness values were measured using a commercial hardness kit (La Motte Chemical Products, Chestertown, MD). Samples of SRNOM and NRNOM at 10 mg/L DOC were measured for absorbance at 515 nm (752W UV–vis Spectrophotometer, Beckman Coulter, Mississauga, ON) according to the Hach 8025 method against choloroplatinate-cobalt standards at pH 7.6.

Table 1
Water chemistry used for toxicity tests and the resulting 48 h EC50 for Ni to *D. pulex*. Water chemistry is shown as mM except for Ni values which are μM and alkalinity and natural organic matter (NOM) which are given as mg/L. The upper (UCL) and lower (LCL) 95% confidence limits of the EC50 values are included. Tests characterizing the effect of Suwannee River natural organic matter on Ni toxicity are labeled as SRNOM while those with Nordic Reservoir natural organic matter are NRNOM. Within a test series, EC50 values labeled with the same letter are not significantly different from each other. All values represent measured concentrations except for SO_4 and HCO_3, which are nominal and toxicity data which are calculated from measured values.

	Ca	Mg	Na	K	Cl	SO_4	HCO_3	pH	DOM	Alk	EC50	LCL	UCL
Ca	0.02	0.14	0.57	0.027	0.027	0.16	0.57	7.85	0.15	28	7.5[a]	5	10
Ca	0.05	0.14	0.57	0.027	0.027	0.19	0.57	7.85	0.15	28	17 [b]	14	21
Ca	0.1	0.14	0.57	0.027	0.027	0.24	0.57	7.85	0.15	28	22 [b]	18	25
Ca	0.18	0.14	0.57	0.027	0.027	0.32	0.57	7.85	0.15	28	16 [b]	14	22
Ca	0.37	0.14	0.57	0.027	0.027	0.51	0.57	7.85	0.15	28	46 [c]	39	56
Ca	0.87	0.14	0.57	0.027	0.027	1.01	0.57	7.85	0.15	28	92 [d]	81	100
Ca	1.25	0.14	0.57	0.027	0.027	1.39	0.57	7.85	0.15	28	89 [d]	79	98
Ca	1.47	0.14	0.57	0.027	0.027	1.61	0.57	7.85	0.15	28	132 [e]	112	152
Mg	0.17	0.01	0.57	0.027	0.027	0.18	0.57	7.85	0.15	28	28 [a]	19	35
Mg	0.17	0.04	0.57	0.027	0.027	0.21	0.57	7.85	0.15	28	30 [a]	23	36
Mg	0.17	0.29	0.57	0.027	0.027	0.46	0.57	7.85	0.15	28	40 [ab]	32	52
Mg	0.17	0.49	0.57	0.027	0.027	0.66	0.57	7.85	0.15	28	30 [a]	26	38
Mg	0.17	0.82	0.57	0.027	0.027	0.99	0.57	7.85	0.15	28	53 [bc]	46	63
Mg	0.17	1.03	0.57	0.027	0.027	1.2	0.57	7.85	0.15	28	51 [bc]	44	56
Mg	0.17	1.44	0.57	0.027	0.027	1.61	0.57	7.85	0.15	28	63 [c]	55	71
Na	0.17	0.14	0.20	0.027	0.20	0.31	0.20	*6.30*	0.15	0.8	26 [a]	22	32
Na	0.17	0.14	0.50	0.027	0.50	0.31	0.20	*6.71*	0.15	0.8	25 [a]	20	28
Na	0.17	0.14	1.0	0.027	1.0	0.31	0.20	*6.81*	0.15	0.8	26 [a]	23	31
K	0.17	0.14	0.57	0.005	0.005	0.31	0.57	7.85	0.15	28	32 [a]	27	37
K	0.17	0.14	0.57	0.12	0.12	0.31	0.57	7.85	0.15	28	42 [a]	36	50
K	0.17	0.14	0.57	0.25	0.25	0.31	0.57	7.85	0.15	28	33 [a]	29	37
K	0.17	0.14	0.57	0.45	0.45	0.31	0.57	7.85	0.15	28	34 [a]	27	41
K	0.17	0.14	0.57	0.78	0.78	0.31	0.57	7.85	0.15	28	33 [a]	27	41
pH1	0.17	0.14	0.02	0.027	0.027	0.31	0.02	5.6	0.15	1	22 [a]	17	26
pH1	0.17	0.14	0.20	0.027	0.027	0.31	0.20	7.6	0.15	10	26 [a]	22	29
pH1	0.17	0.14	0.87	0.027	0.027	0.31	0.87	7.9	0.15	42	24 [a]	18	29
pH1	0.17	0.14	1.00	0.027	0.027	0.31	1.00	8	0.15	*52*	23 [a]	20	25
pH2	0.17	0.14	0.57	0.027	0.027	0.31	0.57	5.6	0.15	28	26 [a]	21	32
pH2	0.17	0.14	0.57	0.027	0.027	0.31	0.57	6.3	0.15	28	24 [a]	14	34
pH2	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.0	0.15	28	37 [a]	32	45
pH2	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.5	0.15	28	54 [b]	47	63
pH2	0.17	0.14	0.57	0.027	0.027	0.31	0.57	8.0	0.15	28	68 [b]	60	79
pH2	0.17	0.14	0.57	0.027	0.027	0.31	0.57	8.3	0.15	28	67 [b]	59	76
SRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	0.5	28	17 [a]	12	22
SRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	10	28	50 [b]	42	65
SRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	19.8	28	48 [b]	40	64
SRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	29.3	28	42 [b]	35	51
SRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	41	28	55 [b]	39	87
NRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	1.53	28	25 [a]	18	38
NRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	2.84	28	28 [a]	23	34
NRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	9.8	28	52 [b]	46	55
NRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	16.5	28	89 [c]	76	100
NRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	22.9	28	86 [c]	80	92
NRNOM	0.17	0.14	0.57	0.027	0.027	0.31	0.57	7.85	35.3	28	120 [d]	114	130

2.4. Calculations, statistics and modelling

The 48 h EC50 values for dissolved Ni were calculated by means of Comprehensive Environmental Toxicity Information System (CETIS, Tidepool Scientific Software) using the observed proportional immobilization of neonates at 48 h and the associated measured Ni concentrations. Within a test series EC50s were considered significantly different when the 95% confidence intervals did not overlap.

The speciation of Ni at the EC50 concentration was calculated for each test using measured water chemistry. Two geochemical modelling software packages were compared: the HydroQual Biotic Ligand Model (HQ-BLM ver. 2.3.3 research mode, downloaded from www.hydroqual.com/BLM) and the Windermere Humic Aqueous Model (WHAM ver 6.0, Tipping, 1998: purchased from Centre for Ecology and Hydrology, UK). The HQ-BLM was used in speciation mode with the standard assumption of DOC having a humic content of 10% and with inorganic carbon entered as the measured alkalinity. WHAM modelling was also done using the same assumption of DOC composition (90% fulvic and 10% humic). Particulate matter and the partial pressure of CO_2 were not included and carbonate was entered as HCO_3^-. A check of inorganic carbon modeling in WHAM was done by comparing calculated alkalinity to measured alkalinity; measurements confirmed model outputs.

Due to its wide acceptance for BLM modeling as well as its ease of use and application, the HQ-BLM was used to develop biotic ligand variables. The stability constants for the competitive interaction of cations (Ca^{2+} and Mg^{2+}) on Ni toxicity were calculated according to the method described by De Schamphelaere and Janssen (2002). Linear regression analysis of free cation activities, Ca^{2+} on Ni^{2+} EC50, in the presence of constant Mg^{2+} was used to generate the slope and intercept variables that were used to develop the matrix equations to derive estimates of $\log K_{CaBL}$ (De Schamphelaere and Janssen, 2002). Similarly, regression variables for the toxicity-mitigating effect of Mg^{2+} activity on Ni^{2+} activity at the EC50 concentration in the presence of constant Ca^{2+} were used to estimate the $\log K_{MgBL}$. The conditional equilibrium constant describing the toxic interaction of Ni^{2+} on the biotic ligand ($\log K_{NiBL}$) was calculated from the mean of the intercepts of the seven individual regression relationships of Ni^{2+} on Ca^{2+}, Mg^{2+},

Na^+, K^+, H^+, SRNOM and NRNOM. These calculated variables were applied within the HQ-BLM to develop a Ni BLM for *D. pulex* in soft water. Predicted total dissolved Ni toxicity output from the model was compared to the measured total dissolved Ni toxicity and through a series of model "runs", the critical value (LA50) was adjusted to provide a "best fit" between measured and modelled EC50 values. For comparative purposes, modeling scenarios were also implemented using the BLM parameters for the acute toxicity of Ni to *D. magna* from Wu et al. (2003; $\log K_{NiBL}$ of 4.0) in combination with the recently published data on the competitive effect of Ca and Mg on Ni toxicity to *D. magna* in soft water ($\log K_{CaBL}$ of 4.2 and $\log K_{MgBL}$ of 3.6, Deleebeeck et al., 2007b). In this modeling exercise the critical value was also adjusted to provide a "best fit" between measured and modelled EC50 values.

3. Results

The results of chemical analyses showed that measured values were always within 7% of nominal. Measured total and dissolved Ni concentrations at 0 and 48 h were within 3% of nominal concentrations and demonstrated that essentially all of the Ni was in the dissolved phase. For other cations, the measured difference between filtered and unfiltered concentrations was within 4%. Dissolved oxygen and temperature of both cultures and test media always met test acceptability requirements (Environment Canada, 1996). Differences in Ni toxicity were tested using $NiSO_4$ and $NiCl_2$. The 48 h EC50 for $NiSO_4$ was 16.9 μM (95% confidence interval (CI) of 14.0–18.5) while that of $NiCl_2$ was 15.9 μM (CI of 13.6–18.2).

Geochemical modelling software was used to derive free ion activity concentrations and two software packages, the HQ-BLM (2.3.3) and WHAM (6.0) compared. For speciation in inorganic solutions (no added NOM) WHAM consistently predicted lower Ni^{2+} concentrations than did the HQ-BLM. WHAM predictions for Ni^{2+} were on average 83% of BLM predictions (standard deviation 0.4%, minimum 73%, maximum 90%, $n = 35$). However, solutions with added NOM revealed much larger differences in predicted Ni^{2+} concentration, in the opposite direction. WHAM predictions in these cases were on average 170% of HQ-BLM predictions (standard deviation 0.8%, minimum 96%, maximum 331%, $n = 9$).

3.1. Effects of Ca, Mg, Na, K, Cl and H on Ni toxicity

Increased Ca or Mg concentrations in test solutions resulted in a significant decrease in Ni toxicity (Table 1). The protective effect of Ca was stronger than that of Mg. When Ca concentration increased from 0.02 to 1.25 mM, the 48 h EC50 value increased by a factor of about 18. When Mg concentration increased from 0.01 to 1.44 mM, the 48 h EC50 value increased by a factor of about 2.3 (Table 1). The regression of the EC50 for Ni vs. Ca waterborne concentration had a slope of 77 (μM increase Ni EC50 per mM Ca, $r^2 = 0.98$) while for Mg, the comparable slope was 24 ($r^2 = 0.89$). Ca concentrations that were lower than in the culture water (i.e., 170 μM) resulted in significant increases of Ni toxicity but this did not occur when Mg concentrations were lower than the culture water (i.e., 140 μM). When compared on a free ion activity basis the competitive interactions between Ca^{2+} and Ni^{2+} as well as between Mg^{2+} and Ni^{2+} were evident (Fig. 1A and B) and regression variables are shown in the figure legend.

Ni toxicity testing with varying levels of NaCl and KCl showed that the 48 h EC50 values were not significantly altered (Table 1, Fig. 2A). The pattern of mortality was different in the two different pH test series. The tests with MOPS to control pH (pH set 2) showed an increase in EC50 value at higher pH values (Table 1). The EC50 value increased 2.6-fold over the range from approximately

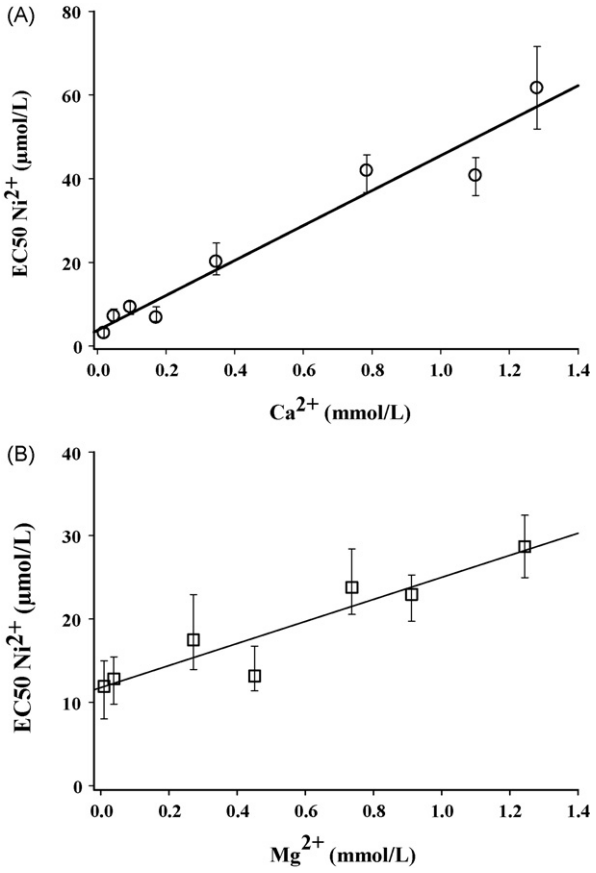

Fig. 1. Protective effect of Ca^{2+} (A) or Mg^{2+} (B) on Ni^{2+} toxicity in soft water. Ni^{2+} EC50 s are shown with 95% CI and ion activities were calculated from measured values using the Hydroqual BLM (ver 2.3.3). The cationic activities were used to derive the linear regression; Ni^{2+} EC50 = 41.6·(Ca^{2+}) + 3.7 ($r^2 = 0.94$, panel A) and Ni^{2+} EC50 = 3.3·(Mg^{2+}) + 11.8 ($r^2 = 0.88$, panel B).

pH 5.6 to pH 8.4. The relationship of H^+ concentration to Ni^{2+} EC50 is given (Fig. 2B) and shows no consistent effect. Regression variables are shown in the figure legend. In pH set 1 the test solutions were prepared so that pH, alkalinity and Na concentration varied. In these tests there were no significant differences in Ni EC50 values (Table 1).

3.2. Effect of NOM on Ni toxicity

The addition of either SRNOM or NRNOM to test solutions resulted in significant increases in the 48 h EC50 for Ni (Table 1, Fig. 3A). The toxicity reducing effect of SRNOM was much weaker than that of NRNOM. The toxicity of Ni was reduced by about 3-fold as DOC concentrations from SRNOM increased from 0.5 to 41 mg/L (Table 1). In the case of NRNOM the increase of DOC concentrations from 1.5 to 35 mg/L resulted in a 5-fold decrease in acute Ni toxicity (Table 1, Fig. 3A). Interestingly, the protective effect of NRNOM was directly related to total dissolved Ni EC50 values while for SRNOM, protective effects did not change significantly above 10 mg/L (Table 1, Fig. 3A). Equally interesting was the observation that in NRNOM solutions the conductivity varied directly with DOC concentration, increasing by nearly 3-fold over the range of tested concentrations. SRNOM solutions did not show this relationship and conductivity was relatively consistent as the concentration of DOC increased. The results of speciation modeling to give the effect of NOM sources on Ni^{2+} toxicity are shown in Fig. 3B. For both SRNOM and NRNOM, consideration of toxicity on a free ion

basis reduced the variability of EC50 values (Fig. 3B). Assessment of colour in SRNOM and NRNOM samples at 10 mg/L DOC indicated that NRNOM solutions were darker, by approximately 2.5-fold in terms of measured colour units.

3.3. BLM development

The model parameters used to describe the toxic interaction of Ni^{2+} on the biotic ligand in the absence of complexation and competition influences as well as the competitive effects of Ca^{2+} and Mg^{2+} were derived from the free ion activity relationships (see slope and intercept variables in the figure legend). This yielded a log K_{NiBL} of 4.87, a log K_{CaBL} of 4.2 and a log K_{MgBL} of 3.6. The development of log K values for Na^+, K^+ and H^+ were not warranted as they either did not alter Ni^{2+} toxicity (Na^+ and K^+, Fig. 2A) or there was an inverse correlation to Ni^{2+} toxicity (H^+ effects Fig. 2B). Modeling was done using the HQ-BLM with two different modified input parameter files, one using the log K values generated by Wu et al. (2003) and Deleebeeck et al. (2007b), shown in Fig. 4A, and the other using the log K values generated by these toxicity studies, shown in Fig. 4B. In both cases LA50 values in the model were adjusted to achieve the best fit. The models provided reasonably good predictions, in the case of the Wu et al. (2003) and Deleebeeck et al. (2007b) model with a critical value of 0.8 and in the case of our data, 5.1 (both as nmol/g wet wt). In both cases, the models predicted the protective effects of Ca^{2+} and Mg^{2+}. The effects of pH (pH set 2) were not well predicted by the model; however, toxicity was over-predicted

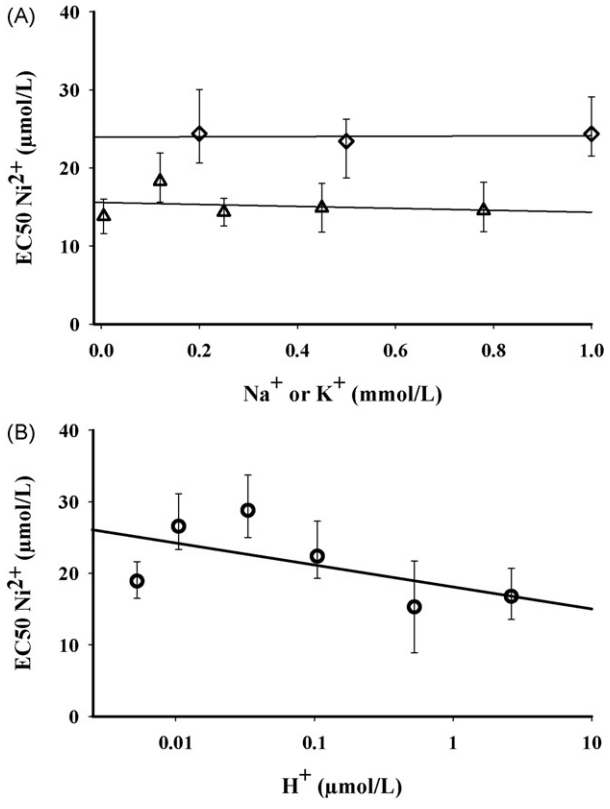

Fig. 2. Effect of Na^+ and K^+ (A) or H^+ (B) on Ni^{2+} toxicity in soft water. Ni^{2+} EC50 values and linear regressions are as per Fig. 1. Note that in panel A, the K^+ test series are denoted with a triangle symbol while Na^+ test series are shown with a diamond symbol. As well, in panel B the data from pH set 2 (MOPS used to adjust test solution pH) is shown. The cationic activities were used to derive the linear regression for each test series; Ni^{2+} EC50 = 0.17·(Na^+) + 24.0 (r^2 = 0.02, panel A), Ni^{2+} EC50 = −1.2·(K^+) + 15.6 (r^2 = 0.05, panel A) and Ni^{2+} EC50 = −3.1·(H^+) + 18.1 (r^2 = 0.34, panel B).

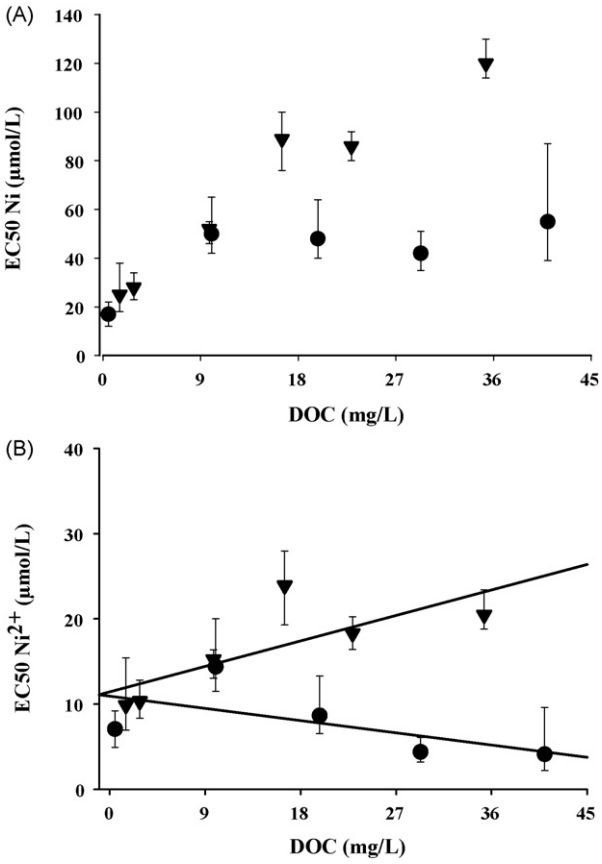

Fig. 3. Protective effect of natural organic matter on Ni toxicity in soft water. Panel A shows Ni toxicity as total dissolved Ni while B shows toxicity as a function of Ni^{2+} concentration. Two different sources of NOM were tested, Suwannee River (filled circle) and Nordic Reservoir (inverted triangles). In panel B the Ni^{2+} activity was used to derive the linear regression of each of the NOM sources; Ni^{2+} EC50 = −0.16·(SRNOM) + 10.9 (r^2 = 0.37), and Ni^{2+} EC50 = 0.58·(NRNOM) + 11.4 (r^2 = 0.58).

(meaning the model offered conservative estimates because predicted EC50 was lower than measured, Fig. 4). The model provided estimates of the protective effects of DOC that were consistent for NRNOM but in both modeling scenarios the predictions were overprotective (Fig. 4). In the case of SRNOM the predictions at low DOC concentrations matched with measured toxicity but higher concentrations did not. This was due to the fact that at low DOC concentrations measured EC50 s increased with increasing DOC but above 10 mg/L DOC there was no change in measured EC50 values (Fig. 4 also see Fig. 3B). Therefore at higher SRNOM levels the BLM predicted reduced toxicity when in fact this did not occur. The BLM predictions of Ni toxicity from the pH set 1 were poor. At low pH the predicted EC50 values were less than measured but at higher pH the EC50 values were much higher. Because pH set 1 tests combined competition and complexation (pH, alkalinity and Na were varied) these data are not shown in Fig. 4.

4. Discussion

Both Ca and Mg protected *D. pulex* from the toxic effects of waterborne Ni (Table 1) and there was a clear competitive interaction between these cations and Ni^{2+}. The fact that both Ca and Mg were found to be protective against Ni toxicity supports the conclusion of other investigators that hardness cations are one of the primary toxicity modifying factors (Pane et al., 2003a; Keithly et al., 2004; Hoang et al., 2004). The protective effect of Ca was significantly greater than that of Mg, a finding which corroborates

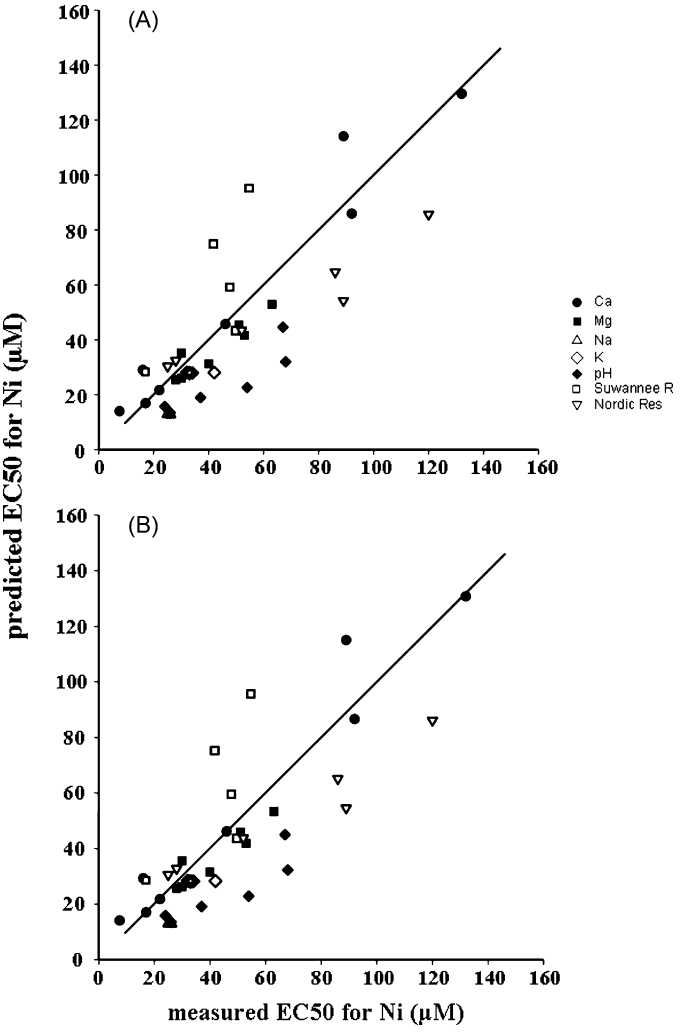

Fig. 4. Measured EC50 values (total dissolved Ni) vs. predicted BLM EC50 output data from two BLM models for the toxicity of Ni in soft water. Panel A shows the fit when modelling parameters developed from Deleebeeck et al. (2007a) and Wu et al. (2003) of log K_{NiBL} of 4.0, log K_{CaBL} of 4.2 and log K_{MgBL} of 3.6 and a LA50 (critical value) of 0.8 nmol/g wet wt are used. Panel B show the fit when applying the modelling parameters developed from the results of this study. In both panels different symbols show different the test series and the line represents the BLM developed for *D. pulex* in soft water (log K_{NiBL} of 4.87, log K_{CaBL} of 4.2 and log K_{MgBL} of 3.6 and a LA50 value of 5.1 nmol/g wet wt). Symbols show different experimental test series (see Table 1) and the solid diagonal line represents the 1:1 line of perfect prediction.

the recently published study of Deleebeeck et al. (2007b) using *D. magna*. Our calculated log K_{CaBL} and log K_{MgBL} were 4.2 and 3.6 while the log K_{NiBL} was 4.87. These values compare to log K_{CaBL} and log K_{MgBL} in Deleebeeck et al. (2007b) of 3.2 and 2.6 for waters of comparable hardness (30 mg/L $CaCO_3$) although it is interesting to note that they calculated values of 4.2 and 3.6 (respectively) in very soft water. Recently the same group has published further on BLM models for the acute toxicity of Ni to *D. magna* in relatively harder waters and in this case the log K_{CaBL} and log K_{MgBL} are 3.1 and 2.47 respectively (Deleebeeck et al., 2008). For the direct effect of Ni^{2+}, our value of 4.87 was higher than the log K_{NiBL} value of 4.0 reported by Wu et al. (2003). The results of this study indicate that *D. pulex* in soft water are both more sensitive to Ni^{2+} and also have a higher degree of sensitivity to the protective effects of Ca and Mg in comparison cladocerans living in hard water. The elevated protective effect of Ca and Mg in soft waters compared to hard waters appears to occur across species and therefore is related to Ca and/or Mg dynamics, as discussed by Deleebeeck et al. (2007b).

The protective effect of Ca and Mg on Ni toxicity likely reflects competition among these cations for uptake sites on *D. pulex*. These sites could be specific for Ca, specific for Mg or more generalized sites where Ni uptake occurs and the effects of Ca and Mg are indirect. In terms of Ni uptake at specific carriers for either Ca and/or Mg, competition at a common trans-membrane uptake site is thought to be due to similarities of ionic diameter and charge (Playle et al., 1993; Schubauer-Berigan et al., 1993; Meyer et al., 1999). The interference with Ca uptake by divalent metals such as Zn is well-characterized (Hogstrand et al., 1994) and elevated levels of metal lead to disruption of internal Ca balance and hypocalcemia. Additionally Ni induced disruption of a Mg uptake pathway is possible, perhaps through the Mg^{2+}–HCO_3^- co-transport pathway (Gunther et al., 1986; Pane et al., 2003a). This theory is supported by the similarity of the ionic radii (Ni–0.066 and Mg–0.069 nm; Weast, 1973). Moreover, Pane et al. (2003a) demonstrated Mg–Ni interactions as a major mechanism of Ni toxicity. Protective effects of Ca and Mg may also be indirect, as a result of the role that Ca plays with respect to membrane permeability (McWilliams, 1983; Hunn, 1985).

A further note worthy of discussion and related to culture conditions is that with very few exceptions the 48 h EC50 values in culture water were always slightly lower than in manipulated test series waters. While this was not the subject of our study, the consistently slightly higher EC50 values from solutions that were very close in composition to culture conditions and for cations which did not demonstrate a competitive interaction with Ni toxicity suggests that a small eustress effect (defined here as an increased resistance to Ni as a result of small changes in water chemistry) was induced by a change in water chemistry. We did not find any influence of Na, Cl or K on Ni toxicity, in agreement with Wu et al. (2003), Pane et al. (2003a, 2004a) and Deleebeeck et al. (2007b) and illustrating that Ni accumulation and effects in *Daphnia* sp. probably do not involve these ion uptake mechanisms.

The influence of pH on Ni toxicity was complex and difficult to interpret. In experiments where MOPs was used to adjust pH the 48 h EC50 values increased as pH increased up to pH 8.0 (Table 1, Fig. 2B). This clearly indicates that there is no competitive interaction between Ni^{2+} and H^+ because toxicity increased as proton concentration increased (i.e., decreased EC50 with decreased pH; Table 1, Fig. 2B). The other test with varying pH showed no (pH set 1) effects on Ni toxicity as pH increased (Table 1). Both HCO_3^- and OH^- have a high affinity for Ni (Tipping, 1994) and in the absence of competitive interactions with H^+ a decrease in toxicity would be expected as pH and alkalinity increase (Wu et al., 2003; Hoang et al., 2004). However, the increased carbonate complexation of Ni^{2+}as pH increased did not reduce toxicity (Table 1, pH sets 1). One interpretation of the data is that increased alkalinity enhanced Ni toxicity, canceling out the expected reduction in Ni toxicity as pH increased. Equally it is possible that changes in HCO_3^- test chemistry had a physiological effect on the daphnids resulting in altered sensitivity to Ni.

Other studies examining the effect of pH on Ni toxicity have shown variable outcomes. For example, some studies are similar to our results in showing that as pH increases (especially between 7.0 and 8.4), toxicity decreases (Reader et al., 1989; Pyle et al., 2002). However, other studies have reported no effect of pH on Ni toxicity (Van Sprang and Janssen, 2001; Hoang et al., 2004). At least one study on the effects of Ni to *Ceriodaphnia dubia*, reported an increase in Ni toxicity when pH increased (Schubauer-Berigan et al., 1993). The study of Deleebeeck et al. (2007a) reported no effect on Ni toxicity to *D. magna* up to pH 7.5 but showed increased toxicity between pH 7.5 and 8.1. Within our pH test series, why variations in pH and alkalinity together did not alter Ni toxicity but pH change alone did (i.e., using the MOPS treatment) remains unclear since

MOPS is considered a buffer that does not complex metals in solution (Kandegedara and Rorabacher, 1999; De Schamphelaere et al., 2004). Our study illustrates that pH effects are not well understood, that the buffering method is important in understanding the influence of pH on Ni toxicity and that pH/alkalinity effects in *D. pulex* in soft water requires further study.

The tests with SRNOM and NRNOM illustrated that Ni toxicity was reduced in the presence of organic matter (Table 1, Fig. 3A). These tests also clearly demonstrated that at concentrations above 10 mg DOC/L the different sources of NOM have different toxicity-mitigating capacities for Ni. NRNOM had a much higher protective capacity than SRNOM and toxicity mitigation was directly related to DOC concentration. SRNOM protected against Ni toxicity up to 10 mg DOC/L however, EC50 values then were constant as tested DOC levels were further increased. A similar result was noted by Hoang et al. (2004) where NOM appeared to saturate, in their case above 5 mg DOC/L. The NOM induced reduction of toxicity was expected as NOM will complex Ni^{2+} (Livens, 1991) and this was evident in our studies (Fig. 3B). Clearly the two sources of NOM differed in their capacity to complex Ni^{2+}. The study of Schwartz et al. (2004) concluded that the relative protective effect of NOM source on metal-toxicity differed and could be estimated based on the spectrophotometric properties (i.e., the absorbance at 340 nm). Darker NOM sources provided a strong protective effect. While our colour measurements were not at this wavelength they did illustrate that NRNOM solutions were darker and SRNOM thus generally supported the conclusions of Schwartz et al. (2004) and suggested that the specific absorbance coefficient principle may extend to Ni. A reduction of Ni toxicity in the presence of elevated DOC has been shown previously in the fathead minnow, *Pimephales promelas* (Wu et al., 2003; Deleebeeck et al., 2007a) and *D. magna* (Deleebeeck et al., 2007b). In general the toxicity reducing impact of NOM is relatively low compared to that reported other metals. In this regard, the study of Wu et al. (2003) explained that the reduced influence of DOC for Ni (compared to other metals) was likely due to the relatively high EC50 concentrations coupled with the relatively low binding constants of Ni–DOC complexes.

The development of two BLMs for *D. pulex* in soft waters showed that toxicity could be predicted accurately over a 16-fold range of toxicity (Fig. 4). Both models predicted remarkably similar toxicity values even though the log K_{NiBL} and LA50 values differed considerably (Fig. 4A and B). The model predictions provided good estimates of toxicity for solutions with varying concentrations of Ca, Mg, Na and K (Fig. 4). Modeling the toxicity of pH changes (without alkalinity changes) was not accurate and those predictions where pH and alkalinity both varied were particularly poor (data not shown on graph). The reason for the poor fit of data when pH and alkalinity varied is due to the model prediction of reduced Ni^{2+} due to carbonate complexation. The model predicts a strong reduction in toxicity when in fact no change occurs. Modeling of NOM was also problematic particularly at elevated levels of SRNOM. In this case the model predicted an increase in EC50 when measured EC50 values remained constant (at DOC concentrations >10 mg/L). Modeling of NRNOM effects results in an underestimation of actual toxicity and shows that the affinity of Ni^{2+} for NRNOM was less than actually occurred.

In conclusion, the results of this study indicate that the hardness cations, Mg and Ca, as well as NOM reduce Ni toxicity to *D. pulex*. In terms of the protective effects of cations, $Ca^{2+} > Mg$ with Na and K having no effect on Ni toxicity. The BLM derived from the *D. pulex* toxicity data (Fig. 4B) and the BLM using model parameters from Deleebeeck et al. (2007b) and Wu et al. (2003; Fig. 4A) both provided good estimates of acute toxicity that accounted for the effects of Ca and Mg competition but not for the effect of changing pH or NOM. The effects of NOM varied depending on source and therefore the BLM, which does not consider different forms, could not account for all NOM of the effects of NOM. The effects of pH and alkalinity were not consistent and require further study.

Acknowledgements

This study included valued contributions from the CANMET-MMSL Analytical Services Group at Natural Resources Canada in Ottawa, particularly R. Karwowska. We also extend our sincere thanks to the CANMET-MMSL Ecotoxicology Lab where this study was conducted and especially staff members M. King, M. Schwartz, S. Krack and Dr. B. Vigneault. Drs. R. Smith and P. Gillis provided assistance for the analysis and interpretation of results and the contributions of Maxxam Analytics Inc., particularly M. Smith and F. Mo, for colour measurements are also acknowledged. Funding for this project was through NSERC via a CRD grant with associated support from the International Lead Zinc Research Organization (ILZRO), International Zinc Association (IZA), International Copper Association (ICA), Copper Development Association (CDA), Nickel Producers Environmental Research Association (NiPERA), Noranda-Falconbridge (now Xtrata), Teck Cominco, and Inco (now Vale Inco Limited). CMW is supported by the Canada Research Chair Program.

References

Anke, M., Angelow, L., Glei, M., Muller, M., Illing, H., 1995. The biological importance of nickel in the food chain. Fres. J. Anal. Chem. 352, 92–96.
Astrom, M., Bjorklund, A., 1996. Hydrogeochemistry of a stream draining sulfide-bearing postglacial sediments in Finland. Water Air Soil Pollut. 89, 233–246.
CCME, Canadian Council of Ministers of the Environment. 1987. Canadian Water Quality Guidelines. Guidelines Division, Environment Canada. pp. 6–43-6-45.
Chowdhury, M.J., Bucking, C., Wood, C.M., 2008. Pre-exposure to waterborne nickel downregulates gastrointestinal nickel uptake in rainbow trout: indirect evidence for nickel essentiality. Environ. Sci. Technol. 42, 1359–1364.
Clifford, M., McGeer, J.C., 2009. Development of a biotic ligand model for the acute toxicity of zinc to *Daphnia pulex* in soft waters. Aquat. Toxicol. 91, 26–32.
David, S.M., Somers, R.A., Reid, R.A., Ingram, R. 1997. Crayfish species assemblages in softwater lakes in seven tertiary watersheds in south-central Ontario. Ontario Ministry of Environment and Energy Report. ISBN 0-7778-6504-1. 100 pp.
Deleebeeck, N.M.E., De Schamphelaere, K.A.C., Heijerick, D.G., Bossuyt, B.T.A., Janssen, C.R., 2008. The acute toxicity of nickel to *Daphnia magna*: predictive capacity of bioavailability models in artificial and natural waters. Ecotoxicol. Environ. Saf. 70, 67–78.
Deleebeeck, N.M.E., De Schamphelaere, K.A.C., Janssen, C.R., 2007a. A bioavailability model predicting the toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) in synthetic and natural waters. Ecotox. Environ. Saf. 67, 1–13.
Deleebeeck, N.M.E., Muyssen, B.T.A., De Laender, F., Janssen, C.R., De Schamphelaere, K.A.C., 2007b. Comparison of nickel toxicity to cladocerans in soft versus hard surface waters. Aquat. Toxicol. 84, 223–235.
De Schamphelaere, K.A.C., Heijerick, D.G., Janssen, C.R., 2004. Comparison of different pH buffering techniques on the toxicity of copper and zinc to *Daphnia magna* and *Pseudokirchneriella subcapitata*. Ecotoxicology 13, 697–705.
De Schamphelaere, K.A.C., Janssen, C.R., 2002. A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effect of calcium, magnesium, sodium, potassium and pH. Environ. Sci. Technol. 36, 48–54.
De Schamphelaere, K.A.C., Janssen, C.R., 2004a. Bioavailability and chronic toxicity of zinc to juvenile rainbow trout (*Oncorhynchus mykiss*): comparison with other fish species and development of biotic ligand model. Environ. Sci. Technol. 38, 6201–6209.
De Schamphelaere, K.A.C., Janssen, C.R., 2004b. Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. Environ. Toxicol. Chem. 23, 1365–1375.
Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P.R., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. 1. Technical basis. Environ. Toxicol. Chem. 20, 2383–2396.
Environment Canada. 1996. Biological test method: acute lethality test using *Daphnia* spp. EPS/RM/11. Ottawa, Ontario 55 pp.
Gunther, T., Vormann, J., Averdunk, R., 1986. Characterisation of furosemide-sensitive Mg^{2+} influx in *Yoshida ascites* tumor cells. FEBS Lett. 197, 297–300.
Hoang, T.C., Tomasso, J.R., Klaine, S.J., 2004. Influence of water quality and age on nickel toxicity to fathead minnow (*Pimephales promelas)*. Environ. Toxicol. Chem. 23, 86–92.
Hogstrand, C., Wilson, R.W., Polgar, D., Wood, C., 1994. Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. J. Exp. Biol. 186, 55–73.

Hunn, J.B., 1985. Role of calcium in gill function in freshwater fishes. Comp. Biochem. Physiol. A 82, 543–547.
Jeziorski, A., Yan, N.D., 2006. Species identity and aqueous calcium concentrations as determinants of calcium concentrations of freshwater crustacean zooplankton. Can. J. Fish. Aquat. Sci. 63, 1007–1013.
Kandegedara, A., Rorabacher, D.B., 1999. Noncomplexing tertiary amines as 'better' buffers covering the range of pH 3–11. Temperature dependence of their acid dissociation constants. Anal. Chem. 71, 3140–3144.
Keithly, J., Brooker, J.A., DeForest, D.K., Wu, B.K., Brix, K.V., 2004. Acute and chronic toxicity of nickel to a cladoceran (*Ceriodaphnia dubia*) and an amphipod (*Hyalella azteca*). Environ. Toxicol. Chem. 23, 691–696.
Livens, R.F., 1991. Chemical reactions of metals with humic material. Environ. Pollut. 70, 183–208.
McGeer, J.C. The biotic ligand model as a tissue residue approach for metals. In: Meador, J. Adams, W.J. (Eds.), Tissue Residue Approach for Toxicity Assessment: Invertebrates and Fish. SETAC Press, Pensacola, FL, in press.
McWilliams, P.G., 1983. An investigation of the loss of bound calcium from the gills of the brown trout, *Salmo truto*, in acid media. Comp. Biochem. Physiol. A 74, 107–116.
Meyer, J.S., Santore, R.C., Bobbitt, J.P., DeBrey, L.D., Boese, C.J., Paquin, P.R., Allen, H.E., Bergman, H.L., Di Toro, D.M., 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free ion activity does not. Environ. Sci. Technol. 33, 913–916.
Muyssen, B.T.A., Brix, K.V., DeForest, D.K., Janssen, C.R., 2004. Nickel essentiality and homeostasis in aquatic organisms. Environ. Rev. 12, 113–131.
Nielsen, F.H., 1996. Other trace elements. In: Zielger, E.E., Filer Jr., L.J. (Eds.), Present Knowledge in Nutrition, seventh ed. International Life Sciences Institute-Nutrition Foundation, Washington, DC, pp. 353–377.
Niyogi, S., Wood, C.M., 2004. The Biotic Ligand Model, a flexible tool for developing site specific water quality guidelines for metals. Environ. Sci. Technol. 38, 6177–6192.
Pane, E.F., Haque, A., Wood, C.M., 2004b. Mechanistic analysis of acute, Ni-induced respiratory toxicity in the rainbow trout (*Oncorhynchus mykiss*): an exclusively branchial phenomenon. Aquat. Toxicol. 69, 11–24.
Pane, E.F., McGeer, J.C., Wood, C.M., 2004a. Effect of chronic waterborne nickel exposure on two successive generations of *Daphnia magna*. Environ. Toxicol. Chem. 23, 1051–1056.
Pane, E.F., Richards, J.G., Wood, C.M., 2003b. Acute waterborne nickel toxicity in the rainbow trout (*Oncorhynchus mykiss*) occurs by a respiratory rather than an ionoregulatory mechanism. Aquat. Toxicol. 63, 65–82.
Pane, E.F., Smith, C., McGeer, J.C., Wood, C.M., 2003a. Mechanisms of acute and chronic waterborne nickel toxicity in the freshwater cladoceran, *Daphnia magna*. Environ. Sci. Technol. 37, 4382–4389.
Paquin, P.R., Gorsuch, J.W., Apte, S., Batley, G.E., Bowles, K.C., Campbell, P.G.C., Delos, C.G., Di Toro, D.M., Dwyer, R.L., Galvez, F., Gensemer, R.W., Goss, G.G., Hogstrand, C., Janssen, C.R., McGeer, J.C., Naddy, R.B., Playle, R.C., Santore, R.C., Schneider, U., Stubblefield, W.A., Wood, C.M., Wu, K.B., 2002. The biotic ligand model: historical overview. Comp. Biochem. Physiol. 133C, 3–35.
Phipps, T., Tank, S.L., Wirtz, J., Brewr, L., Coyner, A., Ortego, L.S., Fairbrother, A., 2002. Essentiality of nickel and homeostasis for its regulation in terrestrial organisms. Environ. Rev. 10, 209–261.
Playle, R.C., Dixon, D.G., Burnison, K., 1993. Cu and Cd binding to fish gills: estimates of metal-gill stability constants and modeling of metal accumulation. Can. J. Fish. Aquat. Sci. 50, 2678–2687.
Pyle, G.G., Swanson, S.M., Lehmkuhl, D.M., 2002. The influence of water hardness, pH, and suspended solids on nickel toxicity to larval fathead minnows (*Pimephales promelas*). Water Air Soil Pollut. 133, 215–226.
Reader, J.P., Everall, N.C., Sayer, M.D.J., Morris, R., 1989. The effect of eight trace metals in acid soft water on survival, mineral uptake and skeletal calcium deposition in yolk-sac fry of brown trout, *Salmo trutta*. J. Fish Biol. 35, 187–198.
Santore, R.C., Di Toro, D.M., Paquin, P.R., Alen, H.E., Meyer, J.S., 2001. Biotic ligand model of the acute toxicity of metals. 2. Application to acute copper toxicity in freshwater fish and Daphnia. Environ. Toxicol. Chem. 20, 2397–2402.
Schubauer-Berigan, M.K., Dierkes, J.R., Monson, P.D., Ankley, G.T., 1993. pH-dependent toxicity of Cd, Cu, Ni, Pb, and Zn to *Cerioaphnia dubia, Pimephales promelas, Hyalella azteca* and *Lumbriculus variegates*. Environ. Toxicol. Chem. 12, 1261–1266.
Schwartz, M.L., Curtis, P.J., Playle, R.C., 2004. Influence of natural organic matter source on acute copper, lead and cadmium toxicity to rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 23, 2889–2899.
Shaw, J.R., Dempsey, T.D., Chen, C.Y., Hamilton, J.W., Folt, C.L., 2006. Comparative toxicity of cadmium, zinc, and mixtures of cadmium and zinc to daphnids. Environ. Toxicol. Chem. 25, 182–189.
Tipping, E., 1998. Humic ion-binding model VI: an improved description of the interactions of protons and metal ions with humic substances. Aquat. Geochem. 4, 3–48.
Tipping, E., 1994. WHAM—A chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. Comput. Geosci. 20, 973–1023.
USEPA (U.S. Environmental Protection Agency). 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012. 273 pp.
Van Sprang, P.A., Janssen, C.R., 2001. Toxicity identification of metals: development of toxicity identification fingerprints. Environ. Toxicol. 20, 2604–2610.
Weast, R.C. (Ed.), 1973. Handbook of Chemistry and Physics, 54th ed. CRC Press, Cleveland, OH, USA.
Wu, K.B., Paquin, P.R., Navab, V., Mathew, R., Santore, R.C., Di Toro, D.M. 2003. Development of a biotic ligand model for nickel: phase I. Water Environment Research Foundation, report 01-ECO-10T.
Zwolsman, J.J.G., van Bokhoven, A.J., 2007. Impact of summer droughts on water quality of the Rhine River—a preview of climate change. Water Sci. Technol. 56, 45–55.