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THE INFLUENCE OF DISSOLVED ORGANIC CARBON (DOC) AND CALCIUM ON THE TOXICITY OF COPPER AND NICKEL TO THE FRESHWATER ALGA *Selenastrum capricornutum* AND THE ZOOPLANKTER *Daphnia magna*

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Thesis submitted to the
School of Graduate Studies and Research
University of Ottawa
in partial fulfillment of the requirements for the
M.Sc. degree in the

Ottawa-Carleton Institute of Biology

ABSTRACT

The toxicity of copper and nickel was measured in algal cultures of *Selenastrum capricornutum* and zooplankton cultures of *Daphnia magna* to determine if dissolved organic carbon (DOC) and/or calcium concentrations can influence toxicity of these two metals. Water samples were collected from the Raisin River (high DOC and high calcium), the St. Lawrence River (low DOC and high calcium), the Ottawa River (medium DOC and calcium) as well as two lakes in Nova Scotia. These were Big Dam West Lake (high DOC and low calcium) and Big Dam East Lake (low DOC and low calcium). The concentration sufficient to kill half the population of *Daphnia magna* after 48h of exposure (LC_{50}), and the inhibition of cell growth of *S. capricornutum* after 72 h of exposure (IC_{50}) were used to determine the sensitivity of these species to copper or nickel toxicity in these 5 water samples.

In the copper algal toxicity tests, I found that the IC_{50} of Raison River water was the highest (90 μg Cu L^{-1}) of the five water samples by a considerable amount. The IC_{50} for BDWL samples (9.6 μg Cu L^{-1}) were not significantly different from that for BDEL water (8.7 μg Cu L^{-1}) even though the DOC values were different. The copper IC_{50} for SLR water was the lowest (0.6 μg Cu L^{-1}). These results show that waters with high DOC are generally more resistant to copper toxicity. The role of calcium was inconclusive for the SLR water had the highest level of Ca but the lowest IC_{50}. The nickel IC_{50} of *S. capricornutum* was highest for RR but that for OR, BDWL, SLR, and BDEL were not significantly different.

The copper and nickel acute toxicity test for *D. magna* showed again that the copper LC_{50} for RR water was the highest, but the copper and nickel LC_{50} for SLR was slightly higher than that for OR water even tough DOC values were lower. The copper and nickel LC_{50} for BDEL water was the lowest and not significantly different from that of BDWL

water. It seemed that both high DOC and high Ca increased protection against copper or nickel toxicity for *D. magna*.

In Part 3, I showed that the protection against copper or nickel toxicity was reduced with UVB radiation even though the total DOC was reduced by only 18%. RR water was exposed to UVB radiation for five and ten days. The toxicity of copper and nickel was measured as the inhibition of growth cells *of S. capricornutum*. Water samples were exposed to UVB radiation equivalent to about a third of summer sun conditions for 5 and 10 days. After this exposure the toxicity of copper was reduced from 90 to 38 to 2 μg Cu L^{-1} at 0, 5 and 10 days respectively. The toxicity of nickel was reduced from 200 to 80 to 60 μg Ni L^{-1} over this period. These results show that the duration of exposure to UVB radiation will determine the ability of DOC to protect organisms against copper and nickel toxicity. These observations also illustrate that the role of calcium is not as significant as that for DOC since there was the same amount of calcium at the end of the UV exposure as at the beginning of the exposure.

The role of EDTA in the toxicity of copper and nickel on *S. Capricornutum* was investigated in part 4. I found that addition of EDTA to the RR sample did not significantly reduce copper toxicity but adding EDTA to BDWL, BDEL, and SLR water increased their IC_{50}s. This showed that EDTA complexed the free copper ions in those samples. The addition of EDTA in the nickel toxicity tests, however, did not significantly increase the Ni IC_{50} for RR, OR, BDWL and SLR water suggesting that EDTA did not chelate nickel as well as it did copper.

RÉSUMÉ

La toxicité du cuivre et du nickel sur l'algue *Selenastrum capricornutum* et sur le zooplancton *Daphnia magna* ont été mesurées afin de déterminer si le carbone organique dissout (COD) et de concentrations de calcium variées influencent la toxicité de ces deux métaux. Les échantillons ont été prélevés de la Rivière Raisin (élevée en COD et en calcium), du Fleuve St. Laurent (faible en COD et élevé en calcium), de la Rivière des Outaouais (moyenne en COD et en calcium), du lac Big Dam West (Nouvelle-Écosse) (élevé en COD et faible en calcium) et du lac Big Dam East (Nouvelle-Écosse) (faible en COD et en calcium). La mortalité de *D. magna* après 48 heures (CL_{50}) d'exposition et l'inhibition de la croissance de cellules de *S. capricornutum* après une expositition de 72 heures (CI_{50}) ont servi d'indicateurs de toxicité du cuivre et du nickel dans les cinq échantillons d'eau.

Les tests de toxicité du cuivre sur *S. capricornutum* révèlent que la CI_{50} de la Rivière Raisin était considérablement plus élevée (90 µg Cu L^{-1}) parmi les cinq échantillons. Aucune différence significative de CI_{50} du cuivre n'a été observée entre l'eau du LBDW (9.6 µg Cu L^{-1}) et du LBDE (8.7 µg Cu L^{-1}) malgré la différence des valeurs de COD. Parmi les eaux échantillonnées, celle du Fleuve St. Laurent avait la plus faible CI_{50} de cuivre (0.6 µg Cu L^{-1}). Ces résultats suggèrent que les eaux ayant une concentration de COD élevée sont généralement plus résistantes à la toxicité du cuivre. Le rôle du calcium demeure cependant moins clair puisque l'eau du FSL avec le niveau le plus élevé de Ca avait la plus faible CI_{50}. La CI_{50} du nickel pour *S. capricornutum* était la plus élevée dans la RR mais aucune différence significative entre RO, LBDW, FSL, LBDE.

Les tests de toxicité aigue du cuivre et du nickel chez *D. Magna* démontrent encore que la CL_{50} du cuivre dans la RR était la plus élevée. Celle du FSL était un peu plus élevée

que celle de la RO malgré les valeurs plus faibles de COD. Les CL_{50} du cuivre et du nickel dans l'eau du LBDE étaient les plus basses mais pas significativement différentes de celles du LBDW. Il semble que des concentrations élevées en COD et en Ca augmentent la protection de *D. Magna* contre la toxicité du cuivre et du nickel.

Dans la troisième partie de cette étude, j'ai démontré que la protection contre le cuivre et le nickel est réduite une fois exposé aux radiations d'UVB même si la réduction du COD total n'était que de 18%. L'eau de la RR était exposée cinq et dix jours aux radiations d'UVB (équivalent au tiers de la radiation estivale pour cinq et dix jours). La toxicité du cuivre et du nickel a été déterminée en mesurant l'inhibition de la croissance des cellules de *S. capricornutum*. Suite à cette exposition, la toxicité du cuivre était réduite de 90 à 38 à 2 $\mu g\ Cu\ L^{-1}$ aux jours 0, 5 et 10 respectivement. La toxicité du nickel était réduite de 200 à 80 à 60 $\mu g\ Ni\ L^{-1}$ sur cette même durée. Ces résultats suggèrent que la durée d'exposition aux rayons d'UVB détermine la capacité du COD de protéger les organismes contre la toxicité du cuivre et du nickel. Ces observations illustrent également que le rôle du Ca n'est pas aussi important que le rôle du COD puisque le montant de Ca mesuré au début et à la fin de l'exposition à l'UVB était pareil.

Le rôle d'EDTA dans la toxicité du cuivre et du nickel chez *S. capricornutum* a été étudié dans la quatrième partie. L'ajout du EDTA à l'eau de la RR n'a pas réduit significativement la toxicité du cuivre mais l'ajout du EDTA aux eaux du LBDW, du LBDE, et du FSL a augmenté leurs valeurs de CI_{50}. Ceci démontre que dans ces échantillons, l'EDTA forme des complexes à partir des ions libres de cuivre. L'ajout du EDTA dans les tests de toxicité du nickel n'a pas augmenté la CI_{50} de la RR, de la RO, du LBDW, et du FSL, suggérant que l'EDTA est un meilleur chélateur du cuivre que du nickel.

ACKNOWLEDGEMENTS

The University of Ottawa provided a friendly and stimulating environment for my M.Sc. studies. The Secondary School Teacher Training Development (SSTTD) Project sponsored by the Indonesian Government provided money for travel, living expenses and other costs. This program gave me the opportunity to study in Canada and learn about its culture and science.

I would like to thank my supervisor Dr. David Lean, my co-supervisor, Dr. Jules Blais and the other members of the advisory committee, Dr. Jon Houseman and Dr. Chakrabharti for their advice and encouragement. Dr. Jeff Ridal introduced me to the bioassays used in this investigation and I also appreciated the kindness shown by his family. Lisa Van Loon provided help in editing my thesis. Thanks to the Agus family who lent me their printer while I was writing my thesis. The students in Dr. David Lean's lab (Bontle, Emilie, Jonathan, Nelson, Valbona and Georgine) were friendly and helpful. I also would like to thank Nouri and John from Dr. Chakrabarti's lab for the WHAM program.

I would especially like to thank my long distance husband, Yos Sudarso and my daughter, Ima who encouraged me. Without your permission, I would have never known Canada. Without your patience I would have never finished my study. Now I am coming back to you...to my real life.

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Definitions and Abbreviations

Acidic functional group: Humic and fulvic acid contain a number of ionizable functional groups to which cations may bind. Examples include carboxyl, carbonyl, ammonium ions, and thiol groups (Aiken et al 1985).

Acute toxicity: tests designed to evaluate the relative toxicity of chemicals to selected organims upon short term exposure to various concentration of the test chemical (Rand 1995)

Affinity: the ability of metal to bind organic carbon.

Aliphatic carbon (C_{al}): Of or pertaining to a broad category of carbon compounds distinguished only by a straight or branched, open chain arrangement of the constituent carbon atoms. The carbon-carbon bonds may be saturated or unsaturated (Aiken et al 1985)

Aromatic carbon (C_{ar}) : Organic compounds that contain a benzene ring or that have chemical properties similar to benzene (Daintith 1996).

Allochthonous: produced outside the system, e.g. outside a lake

Autochtonous: produced within the system, e.g. within a lake

Bioassay: an experiment for estimating the nature, constitution, or potency of the materials or of a process by means of the reaction that follows its application to living organisms (Finney 1978).

Chronic toxicity: tests designed to evaluate the relative toxicity of a chemical to selected organims upon long term exposure to various concentrations of the test chemical (Rand 1995).

Confidence limits (CL): the limits indicate the area or range within which the concentration- response line in 19 of 20 samples (95%) taken at random from the same population under the same condition

Culture: the stock organisms under defined and controlled conditions to produce healthy test organisms.

DOC: dissolved organic carbon, represents all the organic material in solution or in suspension passes through a 0.45 μm filter (Drever 1997). In our experiments the filter was cellulose acetate.

DOM: dissolved organic material. Carbon is about 44% of DOM.

EDTA: ethylenediaminetetraacetic acid ($C_{10}H_{14}O_8N_2$)

Endpoint: variables including time and reaction of the organisms indicate the termination of the test (Environment Canada 1992).

Exposure: the contact reaction between the organisms and chemicals.

Fulvic Acids (FA): The fraction of humic substances that are soluble under all pH conditions (Aiken et. al 1985)

Humic acids (HA): the fraction of humic substances that is not soluble in water below a pH of 2, but become soluble at greater pH (Aiken et.al 1985)

Humic substances (HS): complex mixtures of organic compounds that occur naturally In soils, sediments, and natural waters, where they account for a large fraction of The non living organic carbon (Perdue 1998)

Humin: the fraction of humic substances that is not soluble in water at any pH value (Aiken 1985).

IC50 : concentration estimated to inhibit 50% of the alga growth population in a bioassay experiment.

LC50: concentration estimated to kill 50% of the test population in a bioassay experiment (e.g. mortality of *D. magna*).

K_1 and K_2 : conditional equilibrium constants

Ligand (L): a functional group, ion or moleculer bound to a central atom (e.g. metal) in a complex between chemical species called a coordination complex that can formally be thought of as donating electrons (Missler and Tarr. 1991)

ML : metal complex

M^{z+} : free metal ion

{M-X-cell}: the concentration of the surface complex

Sample control: a treatment that duplicates all the condition s and factors that might effect the result of the investigation, except the specific condition that is being

studied (Environment Canada 1992)

Standard control : a control treatment used to determine the absence of measurable toxicity due to basic test condition e.g. milliq water (Environment Canada 1992).

Toxicity: the potential of test material (or a chemical) to have harmful effects on living organisms.

Toxicity test: a method to evaluate the adverse effect of a chemical on living organisms under standardized and reproducible condition (Rand 1995)

UVB: the radiation within a range wavelength of 290-320 nm.

[] : dissolved species

{}: concentration of surface

^{13}C NMR : Nuclear magnetic resonance

≡X-cell: negatively charge binding sites available on the surface.

Chapter 1

GENERAL INTRODUCTION

The toxicity of copper and nickel to aquatic biota has been examined extensively but a rigorous synthesis of the roles of DOC and Ca^{2+} in modulating their toxicity remains incomplete. It has been shown that each of these metals is toxic to a variety of aquatic organisms including protozoan foraminifera (Bresler and Yanko 1995), phytoplankton: *Scenedesmus sp* (Peterson 1982), *Chlorella sp* (NRC 1981), *Chlamynodomonas reinhardtii* (Xue et. al 1988, Macfie et.al 1994), invertebrates (NRC 1981), and fish (NRC 1981). Biesinger and Christiansen (1972) found that copper, at a concentration of 9.8 $\mu g\ L^{-1}$, was toxic to *Daphnia magna*. Bartlett (1974) found that 50 $\mu g\ L^{-1}$ copper inhibited 50% of the growth (IC_{50}) of *Selenastrum capricornutum*. Nickel killed 50% of the population of *Daphnia magna* (LC_{50}) at a concentration of 320 $\mu g\ L^{-1}$ (Stokes1988) and had a chronic toxicity LC_{50} value of 130 $\mu g\ L^{-1}$. Nickel inhibited 50% of the growth of algae at 500 $\mu g\ L^{-1}$ (NRC 1981).

The free metal ion (FMI) is known to be one of the toxic forms to organisms and has been thought to be a better predictor of metal bioavailability than total metal concentration (Sunda and Lewis 1978, Morel 1983). Metal labile forms (hydroxide, carbonate, sulphate, and phosphate) readily convert to more toxic forms under natural conditions (EPA 1980) and their concentration should also be considered part of the toxic pool (Guy and Kean 1979).

The US Environment Protection Agency (EPA 1980) found that the toxicity of copper and nickel to aquatic biota is modified by the physiochemical properties of the water, including the concentrations of dissolved organic carbon (DOC) and calcium (hardness). The interaction of copper and nickel with these factors alters their speciation, which in turn

influences their bioavailability (Roesijadi and Robinson 1994). The DOC complexation of the free metal ion and competition from Ca^{2+} tend to reduce the exposure of metal (as described by Meyer et al. 1999) in synthetic water by reducing M^{2+} binding to receptor sites on the organism cell. In contrast, Campbell (1995) suggested in his review that the presence of dissolved low molecular weight carbon would enhance metal toxicity.

Freshwater organisms used in bioassays

Selenastrum capricormutum (class: Chlorophyceae, order Chlorococcales, family Selenastraceae) is a unicellular, crescent-shaped freshwater green algae, which can be found in both eutrophic and oligotrophic freshwater environments (Picket-Heap 1975). It is about 40 to 60 μm^3 in size (Blaise et al. 1998). The alga *Selenastrum capricormutum* is used in toxicity tests due to the sensitivity of this algae compared to other test organisms (Nyholm and Petterson 1997, Lewis 1995). In addition, since algae represent primary producers at the lowest level of the food chain, disruption at this level would be expected to effect higher trophic levels.

Daphnia magna (class Crustaceae, order Cladocera, family Dapniidae) is a small freshwater crustacean invertebrate and has been called a water flea. It has a maximum length of 5 to 6 mm (Pennak.1983) and prefers to live in moderately hard water. It is used in water toxicity studies for several reasons. Daphnids are widely distributed in the aquatic environment, are relatively sensitive to contaminants, easy to culture in the laboratory, and play an important role in the aquatic food chain as food source for small fishes (Rand 1995).

The 72 hour *S. caprocormutum* test or 48 h *D. magna* test have been adapted and standardized for toxicological testing (ASTM 1993a, Lewis 1989, Environment Canada

1992) and are internationally used. In combination they provide a robust assessment of the possible impacts of chemicals on the lower food chain.

1.2. Calcium

Calcium is considered to be a basic inorganic element essential for growth of algae (Wetzel 1975). It is essential for maintenance of the structural and functional integrity of the cell membrane. Calcium, along with magnesium, is also a major component of water hardness and carbonate (Babich and Stotzky 1983) and as such provides much of the buffering capacity to counteract hydrogen ions from acid rain. Calcium carbonate ($CaCO_3$) is commonly reported as the hardness. Based on US EPA (1980), LC_{50} of copper and nickel to *D. magna* also increases as water hardness is increased in the acute toxicity test. At concentrations of 45, 99, 207 and 226 mg L^{-1} $CaCO_3$, the acute copper LC_{50} values for *D. magna* were 10, 65, 69, and 200 μg L^{-1} respectively. The acute nickel toxicity values for *D. magna* were 4 960, 2 340 and 1810 μg L^{-1} for 206, 100, and 51 mg L^{-1} $CaCO_3$ respectively.

Many authors have claimed that calcium reduces copper toxicity to fish. As water hardness was increased from 48 to 249 mg L^{-1}, the Cu LC_{50} increased by 2.5 fold in 30-day old fathead minnows (Erickson et al 1995). It is widely accepted that the protective action of hardness results from Ca^{2+} competing with the metal species for binding sites on gill surfaces (Pagenkoff 1983, Erickson et. al 1995). At higher hardness, however, the protective effect was decreased (Erickson et al 1995) due to both Ca^{2+} ion and Cu^{2+} ions competing for binding sites on the DOC in the water sample. Meyer et al. (1999) studied the effect of hardness on the binding of nickel and copper to fish gills and its relation to toxicity. It was concluded that free Cu or Ni ions were not good predictors of biological effects, i.e.

mortality, when water hardness varies. For fathead minnows, the measured concentration of Ni-gill complexation and calculated Cu-gill complexation were approximately constant predictors of toxicity when the concentration of Ca in water increased.

Calcium decreases the toxicity of copper and nickel to filamentous bacteria. Shuttleworth (1991) demonstrated that higher calcium levels caused copper and nickel to be less toxic to filamentous bacteria *Thiotrix*. The protective effect of hardness, as $CaCO_3$, on *C. dubia* was correlated with source food (Belanger et al.1989). Neonates whose parents were fed yeast, cherophyl and trout chow were 1.4 to 1.5 times more resistant to Cu than those whose parents were fed with yeast and cherophyll over the same hardness. It was also observed that the 48-h LC_{50} of Cu to *C. dubia* increased from 35 to 79 $\mu g\ L^{-1}$ at water hardness concentrations of 94 and 170 mg $CaCO_3\ L^{-1}$. These studies also showed that calcium reduced copper and nickel toxicity (Jayaraj et al. 1992, Pellegrini et al. 1993, Wurts and Perschbacher 1994). However, other studies showed that calcium had little or no effect on metal toxicity (Lauren and McDonald 1986, Karen et al 1998). Lauren and McDonald (1986) found that the addition of calcium within the range of 25 – 1000 μM had no significant effect on the short-term Cu toxicity toward fish. They hypothesized that toxicity was related to alkalinity, pH and acclimation of test organisms in water exposures. Bury et al (1999) found that increasing Ca^{2+} over the range of 50 to 2 000 μM resulted in only a slight, non-significant, change in the Ag LC_{50} for both fathead minnows and rainbow trout. The low protection by calcium was probably a consequence of the stabilizing effect of Ca^{2+} on the permeability of the fish gill.

1.3 Dissolved Organic Carbon

DOC levels in rivers vary with discharge, the nature of the vegetation in the river basin, climate, and the size of the rivers (Thurman, 1985). In lakes, DOC varies with biological productivity (Drever, 1997), catchment area and the presence of wetlands (Rasmussen et al. 1989). DOC originates from within the aquatic system (autochthonous) and from the catchment (allochthonous).

The normal concentrations of DOC in rivers are 2-15 mg C L^{-1}, with a mean of approximately 5 mg C L^{-1}. However, waters draining wetlands have DOC levels ranging from 5 to 60 mg C L^{-1} with a mean of about 25 mg C L^{-1} (Thurman.1985). In oligotrophic lakes, the DOC concentration is about 1 to 3 mg C L^{-1} but in eutrophic lakes it is typically 2 to 5 mg C L^{-1} (Thurman 1985).

Previously, humic substances were thought to constitute 50 to 90% of DOC (Hayes 1989), but NMR revealed that it makes up only 20-25% (McKnight and Perdue 1998). Aquatic humic substances (HS) consist of fulvic acid (FA), humic acid (HA) and humin, operationally defined using an extraction scheme from soil science. HA is insoluble at low pH, FA is soluble under all pH, and humin is insoluble under any pH values (Aiken et al 1985). In general, FA is comprised of lower molecular weight compounds (500 to 2000 daltons) than HA (2000 to 10^6 or more daltons) (Aiken 1985, Gaffney 1996). Dissolved FA is 40-60% of the DOM of many aquatic systems. (McKnight and Aiken 1998).

The important elements of humic substances are carbon (40-60%), oxygen, 4-5% hydrogen (30-50%), nitrogen (1-4%), sulfur (1-2%) and phosphorus (0.04%) (Gaffney 1985). The chemical structure of humic substances is not well characterized since it differs for each system. The general structure of humic substances has aromatic or aliphatic carbons with a long carbon chain linked by oxygen and nitrogen with carboxylic or phenolic groups as the

major functional groups (Drever 1997). Using ^{13}C-NMR, it was shown that carboxylic acids are the major acidic functional groups in FA and HA and the likely sites for Cu^{2+} and Ni^{2+} binding.

The structure of fulvic acid (FA) contains more aliphatic carbon (C_{al}) and less aromatic carbon (C_{ar}) than humic acid (HA). FA is richer in carboxylic acid, phenolic, and ketonic groups (Gaffney 1996, Drever 1997). In rivers, the dominant structures of humic substances are C_{al}. Only 16-20% of the carbon in FA is C_{ar} with carboxyl acid as the major functional group, and one nitrogen atom per molecule. The C_{al} component is considered more biodegradable than that of C_{ar} and C_{xs} (Perdue.1998). Approximately 30% of the carbon in HA is C_{ar} containing phenolic, carbonyl and carboxyl functional groups and two or three nitrogen atoms per molecule (Malcolm 1985). On the other hand, lake FAs are richer in carboxylic and phenolic acid than lake humic acids (Steinberg and Muenster 1985).

Sun et. al (1997) studied the relationship between composition, structure and bioavailability of DOM. Bacterial growth is positively correlated with the ratio of C_{al} to C_{ar} and the ratio of C to N but negatively correlated with -COOH groups (Perdue 1998).

1.2.1 Metal-DOC association

Differences between structure and binding capacities may exist between humic substances extracted from different water systems (O'Driscoll 1999). The affinity for Cu-fulvic acid complexation is more important than the number of binding sites in determining the ability of DOC to complex metal (McKnight et al 1983) because weak Cu-fulvic acid binding sites easily release free metal ions into the solution. Moreover, the molecular weight

of DOC may be important to metal toxicity since copper bound to low molecular weight organic compounds may also be available to organisms.

Sunda and Lewis (1978) observed that the decreasing of copper ($CuSO_4$) toxicity to unicellular alga, *Monochrysis lutheri*, was due to the increased binding of copper by natural organic ligands from seawaters. They found that the decreased copper toxicity and the increased complexation were due to the dependence of toxicity on free cupric ion. Daly et al. (1990) demonstrated that natural DOM (7 to 8 and 10 to 12 mg C L^{-1} DOC) in 14 to 17 mg L^{-1} $CaCO_3$ reduced the toxicity of copper to freshwater shrimp, *Paratya australiensis*. Hollis et al. (1996) also demonstrated the protective effect of DOM on copper. DOM (5 mg C L^{-1} DOC) kept 0.5 μM of Cu off the gills for 9 days and no fish died, while 0.4 μM Cu without additional DOM caused fish to die within 7 days. This protective effect might be due to free copper as toxic species binding with DOM, thus, less Cu in solution was available to bind to the gill epithelium.

Kim et al. (1999) demonstrated that the increased Cu-LC_{50} for *C. dubia* was higher in equilibrated-DOM solution than in unequilibrated-DOM solution. It was suggested that the reaction of DOM with copper is not fast, and therefore equilibration is needed to increase the survivability of *C. dubia*. Bresler and Yanko (1995) observed that DOC derived from seaweeds reduced the acute toxicity of copper to detached foraminifera *P. spinigera*. The EC_{50} for detached foraminifers was inceased from 1.4 to 8.9 μM Cu with a 50% increase in DOC, whereas the EC_{50} for intact foraminifers was 3.05, 19.49 and 31.33 μM Cu for a 0, 50 and 100% increases in DOC respectively. It is likely that the acid mucopolysacharides covering their cytoplasmic bodies could bind cationic compounds, including metal ions. Erickson et al. (1996) observed that increasing DOM to 5 mg C L^{-1} DOM from 1 mg C L^{-1}

increased the total copper LC_{50} for the fathead minnow four- fold. These studies support the free ion activity model, suggesting that the toxicity of the metal is determined by the concentration of the free or aqueous ion (Morel 1983) since metal-DOC complexation reduces the amount of free ion in aqueous solution.

Other studies found that DOM complexed copper enhanced copper toxicity in natural waters (Giesy et al 1983, Borgman and Carlton 1984, Tubing et al 1992). It was concluded that there was a bioavailable form of complexed copper and that this labile copper fraction was an appropriate measure of copper toxicity.

The relationship between complexation and metal toxicity in natural waters was not easily to obtain because it was difficult to define the organic ligands in natural waters (Guy and Kean 1979). Weak organic complexes (CuL) lead to copper toxicity since they can release the cupric ion to bind with the cell site (CuL + site $\rightarrow$ Cu-site + L). In addition, based on computer model calculations, the free cupric ion was not the only metal species which was toxic; copper-triethylentetraamine ($Cuen^{2+}$) and copper citric acid ($CuHcit^{2-}$) seemed to be toxic, too. Borgman and Ralph (1983) demonstrated that certain species such as copper/amino acid complexes (copper/β alanin complex) and copper/Tris complexes were toxic. Furthermore, Borgman and Carlton (1984) observed that the EC_{50} for total copper concentration in canal water (1.52 μM) was greater than that for inorganic media (0.56 μM) and lake water (0.87 μM) for *D. magna*. The addition of Tris to the samples resulted in a change in the EC_{50} for total copper concentration. The EC_{50} of total copper concentration in inorganic medium (4.18 μM) is higher than that in canal (4.05 μM) and lake water (2.29 μM). They concluded that the toxicity of copper was not only due to the concentration of free copper ion but also to the copper complexing agents present in natural waters. They

suggested that copper complexed with natural complexing agents from either lake or canal water had a greater toxicity than did the copper-Tris complex. In addition, they suggested that this phenomenon could happen when natural water was used for the toxicity test because of the unknown complexing agents in the natural water.

1.3.2 Effect of Calcium and DOC on metal toxicity

The effect of both DOC and calcium on metal toxicity to aquatic biota in water exposure has been investigated. Welsh et al (1994) observed the copper toxicity to larval fathead minnows reduced with increasing DOC in natural, soft lake water. Pentinen et al (1998) suggested that the reduction of cadmium toxicity to *D. magna* was due to Cd – DOC complexation. They observed that Cd was less toxic at the original hardness (0.1 mmol Ca+Mg) of humic lake water (DOC 19 mg C L^{-1}) than in reference water (less than 0.2 mg C L^{-1} of DOC). Increasing hardness (by adding Ca) resulted in an increase in the toxicity of Cd to a level similar to the Cd toxicity in reference water. It suggests that DOM in soft water had a protective effect since DOC complexed the bioavailable Cd. In hard water, Ca^{2+} effectively competed with Cd for available binding sites on the DOM resulting in an increase in Cd toxicity. This complicates the simple idea of Ca ions competing with Cd on membrane sites of organisms and reducing toxicity.

Giesy et.al (1983) demonstrated that in soft acidic water, an increase in TOC reduced the copper LC_{50}. The 48-h copper LC_{50} of pond water collected in April (12.4 mg C L^{-1} of TOC) was 43 μgL^{-1} (0.68 M). It was reduced to 16 μgL^{-1} for samples collected in October (15.6 mg C L^{-1} of TOC). This suggests that there were some stressors present in the natural water sample in autumn causing copper to be more toxic.

Winner (1984) studied the interaction between humic acid and water hardness on bioaccumulation and chronic copper toxicity. He observed that hardness had little effect on either the acute or chronic toxicity of copper. The effect of HA on *D. pulex* was very similar for soft and medium water but was decreased in hard water. Copper was more toxic in hard waters than in either soft or medium water. He suggested that this phenomenon was probably due to the displacement of Cu^{2+} from HA by the increased Ca^{2+} in the water samples. In addition, O'Shea and Manchi (1978) observed the effect of hardness (Ca) on the interaction between trace metals and organic complexing agents under stripping voltametric simulation of natural aquatic conditions. The labile interaction between copper and organic carbon increased with increasing pH. At increased pH and in the presence of calcium, the labile copper humic acid complexation released copper into solution allowing Ca to bind to the humic acid. Mandal et al (2000) observed the competition of Ca^{2+} with Ni^{2+} for binding by a characterized fulvic acid at pH 8±0.1 in artificial lake water and found a trend of increasing labile fraction of Ni^{2+} in the presence of increasing Ca^{2+} concentrations.

Total organic carbon was a more important variable than hardness for the acute copper toxicity test for rainbow trout and fathead minnow. In the acute nickel toxicity test, however, hardness was the best predictor of the LC_{50} in aquatic organisms (US EPA 1980). In the present study, the influence of calcium and DOC that is found in the natural waters (rivers and lakes) on the toxicity of copper or nickel *to S. capricornutum* and *D. magna* was observed. The toxicity of metals in natural waters needs clarification so that we can better manage this resource (Daly et al 1990).

1.3.3 The effect of UV B radiation on DOC

Through ozone depletion, UVB radiation (280-320 nm) is having a greater effect on aquatic ecosystems (Scully and Lean 1994). Some lakes are losing the sunscreen provided by DOC (Schindler et al 1997, Lean 1998) since solar radiation significantly alters the structure of DOC in natural water (Miller 1994). Degradation of DOC includes bleaching effects on coloured DOM in the water body (Hongve 1994), an alteration of DOM from higher molecular weight fraction to lower molecular weight fraction (Strome and Miller 1978, Allard et al 1993, Lindell et al 1995, Frimmel 1998), and increased dissolved inorganic carbon (Miller 1991, Allard et al 1993, Lindell 1996). Thus, the UVB radiation indirectly affects metal speciation and influences the life of aquatic organisms in the ecosystem. The current study observed the alteration of DOC through UVB exposure and subsequent changes in copper or nickel toxicity.

1.4 Ethylenediaminetetraacetatic acid (EDTA)

Ethylenediaminetetraacetic acid (EDTA) is a synthetic chelating agent often used in media for alga growth. EDTA increases the availability of iron while at the same time reducing the uptake of other metals. Since EDTA chelates metal, its use in a metal toxicity test is questionable (Lewis 1996). Environment Canada (1991) suggested adding 300 μgL^{-1} Na_2EDTA for alga growth testing, but US EPA suggested not using this method. The structure of Na_2EDTA is in figure 1.2.

There are limited studies on the role of EDTA in modulating metal toxicity in the presence of natural organic matter. Because of that, an additional purpose of this study is to investigate the role of EDTA in the toxicity of copper and nickel to *S. capricornutum* when natural organic matter is present.

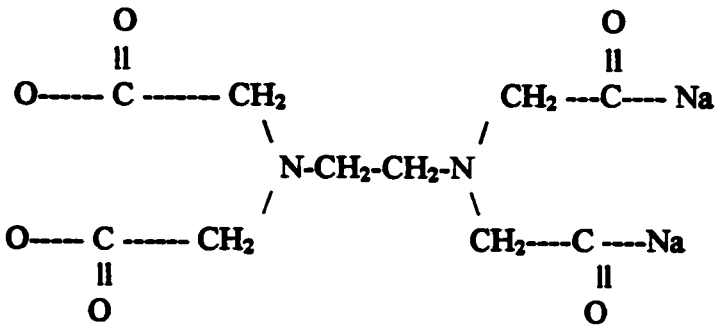


Figure 1.2 Na_2EDTA

1.5 Objectives of this study

This thesis has three objectives:

1) to investigate the influence of DOC and calcium on the toxicity of copper and nickel to freshwater alga *S. capricornutum* and the zooplankter *D. magna*. I predict that water samples with high DOC and Ca will be less sensitive to copper or nickel toxicity.
2) to investigate the influence of UVB on the alteration of DOC and the subsequent effect on copper and nickel toxicity. I predict that UVB exposure will reduce DOC level of water samples, and increase the copper and nickel toxicity.
3) evaluate the role of EDTA on the toxicity of copper and nickel on the alga *S. capricornutum*. I predict that EDTA will reduce copper and nickel toxicity.

Chapter 2

The Influence of Dissolved Organic Carbon (DOC) and calcium (Ca) on the Toxicity of Copper or Nickel on the Freshwater Alga *Selenastrum capricornutum* and Zooplankter *Daphnia magna.*

1. Introduction

Calcium (Ca) and dissolved organic carbon (DOC) are thought to play a role in copper and nickel speciation, thus, influencing the toxicity of these metals. The protective effect of calcium ions indicates that Ca^{2+} was successful in competing with copper or nickel for binding sites on the cell walls of organisms (US EPA 1983, Pagenkoff 1983, Suttlewoth 1991, Jayaraj et al 1992, Pelegrini et al 1993, Erickson et al 1995, Meyer 1999). However, other studies demonstrated that calcium had little or no effect on reducing metal toxicity (Daniel 1998, Laurer and McDonald 1998, Bury et al 1999).

The addition of dissolved organic material (DOM) increased the copper LC_{50} (Sunda and Lewis 1978, Daly 1990, Bresler and Yanko 1995, Erickson et al 1995, Hollis et al 1996, Kim 1999). However, other studies found that the increase in organic carbon enhanced copper toxicity (Guy and Kean 1979, Borgman and Ralp 1983, Giesy et al 1983, Borgman and Carlton 1984, Tubing et al 1991). The addition of natural organic carbon was a challenge since the presence of unknown ligands with unknown structure makes it difficult to predict metal toxicity.

The influence of both DOC and calcium on metal toxicity to aquatic biota (fish and daphnia) in water exposure experiments has been studied previously. Welsh et al. (1994) observed a reduction in copper toxicity to larval fathead minnows with an increase

in DOC in natural soft lake water. Pentinen et al (1998) suggested that DOM in soft water had a protective effect since DOC complexed the bioavailable Cd. However, in hard water DOM increased the Cd toxicity since Ca^{2+} effectively competed with Cd for available binding sites on the DOM, resulting in an increase in Cd toxicity. Giesy et.al (1983) demonstrated that in soft acid water, the increase in TOC reduced the copper LC_{50} on *Simocephalus serrulatus* (daphnidae). Suggesting that there were some stressors present in the natural water sample causing copper to be more toxic.

By using synthetic water, Winner (1984) studied the interaction between humic acid and water hardness on the bioaccumulation of copper by *D. magna* and the chronic toxicity of copper to *D. pulex*. He observed that copper was more toxic in hard water than in either soft or medium water. In addition, O'Shea and Manchi (1978) observed the effect of hardness (Ca) on the interaction between trace metals and organic complexing agents under stripping voltametric simulation of natural aquatic conditions. The labile interaction between copper and organic carbon increased with increasing pH. At increased pH, and in the presence of calcium, the labile copper humic acid complex released copper into solution. Many studies have investigated the effect of calcium and DOC by adding calcium or commercial humic acid to synthetic or natural water. The current study uses natural waters differing in calcium, and DOC levels but with consistent methods are used to assess copper or nickel toxicity. In addition, employing *S. capricornutum* to observe the influence of DOC and Ca on copper and nickel toxicity makes this study different from the previous ones.

The main objective of this study is to investigate the interaction of calcium and dissolved organic carbon (DOC) on the toxicity of copper or nickel to freshwater

organisms, alga *Selenastrum capricornutum* and zooplankter *Daphnia magna*. I predicted that water with high DOC and high calcium levels will have a protective effect against copper or nickel toxicity to *S. capricornutum* or *Daphnia magna*; whereas, water with low DOC and Ca are less protective against copper or nickel toxicity.

2. 2 Study area

Water samples were collected from five (5) different locations differing in their levels of dissolved organic carbon (DOC) and calcium:

1. Raisin River (RR), Cornwall, ON, has high DOC (24.4 mg C L^{-1}) and high calcium levels (86.5 mgL^{-1}),
2. Ottawa River (OR), Ottawa, ON, has high DOC (10 mg C L^{-1}) and mid-calcium levels (15 mgL^{-1}),
3. Big Dam West Lake (BDWL), Kejimkujik, Nova Scotia, has high DOC (10.5 mg C L^{-1}) and low calcium levels (0.641 mgL^{-1}),
4. St. Lawrence River (SLR), Cornwall, ON, has low DOC levels (3.5 mg C L^{-1}) and high calcium (37.2 mgL^{-1}),
5. Big Dam East Lake (BDEL), Kejimkujik, Nova Scotia, has low DOC (3.7 mg C L^{-1}) and low calcium levels (0.606 mgL^{-1}).

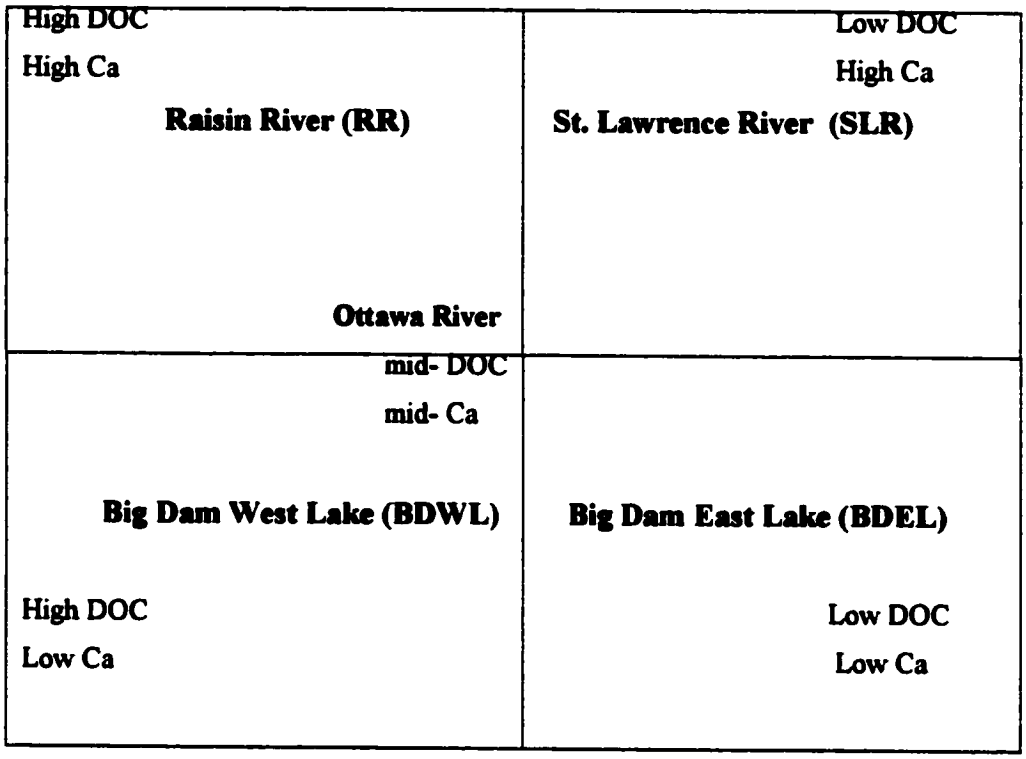


Figure 2.1 tetragonal schema of study areas.

2.3 Materials and Methods

2. 3.1 Experimental Waters and Chemicals

Water samples were filtered using 0.45 μm filters (Sartorius, cellulose acetate) and stored at 4°C in teflon bottles, wrapped with aluminum foil until the start of experiments. Teflon bottles were soaked in 10% HNO_3 for a week, followed by a five-time rinse with milli-Q water then soaked in milli-Q water until they were used (Mandal 1999). The pH was measured (307 Corning pH/temperature) before exposure. Since it could have an effect on the osmotic stress of these organisms, samples where the pH was below 6 (BDWL and BDEL, Kejimkujik waters) were adjusted to 6.5 - 7 for the *Selenastrum* test (Joubert, G. 1983) and to 7 - 8 for the Daphnia test with addition of 0.1

mmol/L NaOH before experiments (Pentinen et al 1998). The adjustment of pHs was done since the pH range for the life of *S. capricornutum* is 6.5-8.5 (Environment Canada 1992) and *D. magna* is 7 –8.5 (Sergy 1990).

The stock solutions of copper and nickel were prepared from their salts ($CuSO_4.5H_20$; 98%-102% chemical purity, BDH and $NiSO_4.6H_2O$; 98-102% chemical purity, ACS). In order to reach chemical equilibrium, test concentrations were prepared by adding the stock solutions to the experimental waters approximately 20 to 24 hrs before organisms were exposed.

2.3.2 Procedures for culturing test organisms and experimental design

2.3.2.1 *Daphnia magna*

Culture.

All test organisms were cultured in the laboratory at the University of Ottawa Biology Department. *Daphnia magna* (were obtained from the St. Lawrence River Institute of Environmental Science, Cornwall, ON) were cultured following established methods recommended by Environment Canada (Reference Method EPS 1/RM/14 1990). Ten Daphnia were cultured in each 1.5-L jar. The jars were cleaned daily. The culture was kept under approximately 800 lux of light intensity, 16 and 8 ± 1 h light and dark, 20 ± 2 °C, and pH 7.5 to 8.5. The hardness and alkalinity of culture medium were adjusted with 192 mg $NaHCO_3$, 120 mg $CaSO_4$, 120 mg $MgSO_4$, 8 mg KCl and 160- 180 mg $CaCO_3$ per liter distilled water. The culture medium was changed every couple of weeks, and its pH was checked three times a week after feeding. Daphnids were fed three times a week with green algae *Selenastrum capricornutum* and *Chlorella sp,* as their primary

food and combined YCT (yeast, cherophyll and Trout chow) as a complement food (Environment Canada 1990). The first neonates were born after 7-12 days of culturing. The second and following neonates were used for testing.

Experimental design.

Experiments were conducted under the same temperature and light intensity as described above. Ten neonates (< 24 hours old) were exposed to 25 ml water samples spiked with Cu as $CuSO_4$ (500, 160, 50, 16, and 5 μgL^{-1}) or Ni as $NiSO_4$ (1000, 320, 100, 32, and 10 μgL^{-1}). Since there was no lethal effect with some of the water samples (RR, SLR), copper or nickel was added in higher concentrations. Three replicates were prepared for each test. The mortality was observed after 48 hrs. No mortality was observed in natural water only or reference water/control only. The mortality of the daphnids was measured as the absence of heartbeat as determined by observation under dissecting microscope. The health of Daphnia (the number of eggs in her bodies), pH before and after tests and dissolved oxygen before and after the tests were all controlled.

2.3.2.2 *Selenastrum capricornutum*

The Selenastrum capricornutum toxicity test was modified from the established methods recommended by Environment Canada (Report EPS1/RM/25 1992).

Culture medium.

The Algal Assay Procedure (AAP) medium developed by the EPA (1978) used in these experiments is composed of macro and micronutrients. These nutrients are essential

to ensure proper algae growth during the test incubation period. The chemical composition of the medium is presented in Table 2.2. The medium was adjusted to final pH of 7.5± 0.1 with 1mM NaOH/HCl. The growth medium was filtered using a sterile apparatus and a 0.2 µm poretics membrane filters (polycarbonate, AMD manufacturing). 0.5 mL of *Selenastrum capricornutum* (Biological Supply Co, Burlington) was inoculated into 50 mL of growth medium in 250-mL glass erlemeyer flask. The inoculated growth medium was incubated at room temperature and under a continuos cool white fluorescent light of 4 klux with shaking at 100 rpm (Gyrotory shaker model G2).

The enriched medium

The concentration of enriched medium culture is 13.75 x alga growth culture without addition of $Na_2EDTA.2H_20$. Enriched medium is used for inducing growth of alga in microplates.

Table 2.1 Final concentration of macro and micronutrients in the growth medium

Chemical	Quantity (mgL^{-1})
A. Macronutrients:	
1. Nitrate sodium ($NaNO_3$)	25.5
1. Magnesium sulfate ($MgSO_4.7H_2O$)	14.7
2. Potassium phosphate (K_2HPO_4)	1.044
3. Sodium bicarbonate ($NaHCO_3$)	15
4. Magnesium chloride ($MgCl_2.7H_2O$)	10
6. Calcium chloride ($CaCl_2.2H_2O$)	~~15~~ 4.42
B. Micronutrients:	(μgL^{-1})
1. Boric acid $(H_3BO_3)_3$	185.52 H_3BO_3
2. Manganese chloride ($MnCl_2.4H_2O$)	415.62 $MnCl_2 \cdot 4H_2O$
3. Zinc chloride (ZnCl)	3.28 $ZnCl_2$
4. Iron chloride ($FeCl_3.6H_2O$)	160 $FeCl_3 \cdot 6H_2O$
5. Cobalt chloride ($CoCl_2.6H_2O$)	1.43 $CoCl_2 \cdot 6H_2O$
6. Sodium molybdate ($NaMoO_4.2H_2O$)	7.26 $NaMoO_4 \cdot 2H_2O$
7. Copper chloride ($CuCl_2.2H_2O$)	0.012 $CuCl_2 \cdot 2H_2O$
8. Sodium ethylenedinitrotetracetic acid ($Na_2EDTA.2H_2O$)	300 $Na_2EDTA \cdot 2H_2O$

please as protocol

Alga inoculum.

Alga cells used for testing were harvested at 4 days post incubation during the exponential alga growth phase (Peterson.1982). The harvested cells were centrifuged (JC 1500) at rotor 7.5, speed 1500 rpm's for 15 minutes The supernatant was discarded and the cells resuspended in $NaHCO_3$. It was centrifuged, the supernatant decanted and it was resuspended in $NaHCO_3$ again. The harvested cell suspensions were diluted in $NaHCO_3$ to have 10 000 cells mL^{-1}. $NaHCO_3$ was used to suspend the cell harvested to avoid plasmolysis of the cells.

Experimental Design

96- well microplates, a scaled down version of the standard US EPA algal bottle (Environment Canada 1993), were used to incubate *Selenastrum* cells spiked with different copper or nickel concentrations. Water samples were spiked with copper (500, 315, 200, 125, 80, 50, 31.5, 20, 12.5, 5 μgL^{-1}) or nickel (1000, 630, 400, 250, 160, 100, 60, 40, 30, 10 μgL^{-1}) 24 h prior to experiments. Aliquotes were placed into each well of the microplates. Additional concentrations of copper and nickel were used in between the concentrations where large differences in cell yields where observed near the IC_{50} value.

Each well of microplates contained 200 µL of the test solution (samples spiked with copper or nickel), 10 µL of the enriched-medium, and 10 µL of the fourth-day aged alga inoculum. Every concentration was replicated 4 times. Ten replicates of the natural water plus enriched medium without spiked copper and nickel were used as control sample for the variability in alga growth for each concentration. 10 replicates of milli-Q

water plus enriched medium and no spiked copper or nickel were used as control standards.

The microplates were removed from their individual packing immediately before use. The lids were placed on the microplates and each microplates was sealed with clear plastic. The microplates were incubated at room temperature (22°C), under continuous cool white fluorescent light at an intensity of 4 klux in 100-rpm shaker for 72 hr.

Cell count

A Neubaueur counting chamber (hemacytometer) was used for algal cell enumeration (Blaise et al 1996). Micropipetting techniques (1-1000 µL) were used to deliver aliquots to the Neubaueur counting chamber. The cell were counted under compound microscope (Zeis) focused at 100 x magnification (10x ocular with a 10x objective).

2.4 Statistical analysis

2.4.1 48-h *Daphnia magna* test:

LC_{50} estimates values and 95% confidence limits were calculated by probit analysis (LC_{50} calculation software programme, Harras et. al 1986). A Tukey multiple comparison with a one way ANOVA was used to compare the LC_{50} values and confidence limits among location (Zar 1999). The calculations were performed by Systat® 7.01 software package for windows. (Wilkinson 1997)

2.4.2 72-h *S. capricornutum* test:

The cell yield of control in the same sample statistically were compared by using a nonparametric Mann-Whitney-U test (Zar 1999) Then, alga growth at each concentration of copper and nickel for each sample were calculated. The IC_{50} (the 50% inhibition growth) was performed by using the following equation (Environment Canada 1991):

I = (Rc-R)/Rc x 100, where:

I : percentages of algal growth inhibition

Rc: the mean cell yield of control sample.

R : cell yield for each test concentration.

IC_{50} estimates values and 95% confidence limits were calculated by probit analyses (LC_{50} calculation software program, Harras et al.1986). Comparison of the IC_{50} and their confidence limits among location was analysed with one way ANOVA with Tukey multiple comparison (Systat® 7.01. software package for windows)

2.4.3 Sensitivity of metal and organism.

A comparison of toxicity levels between copper and nickel, and the sensitivity of the organism between *S. capricornutum* and *D. magna* were analysed by Kruskal - Wallis using one way analyses (Zar 1999) and performed by Systat® 7.01. software package for windows.

2.5 Results

2.5.1 The 72 h *S. capricornutum* tests

Control Standard

The control standard for the experiments was milli-Q water plus enriched medium without EDTA addition. After three days incubation the alga cell yield was 18 times greater than the initial algal cell (10 000 cells mL^{-1}). The average of the standard control for all experiments was 180 000 cells mL^{-1}

Control growth of S. capricornutum.

The control sample of algal growth for each experiment was the water sample plus enriched medium without copper, nickel and EDTA. Figure 2.1 illustrates that the control sample for algal growth for the copper and nickel are identical at each location. The highest algal cell yields was for the RR and the OR water. The number of algal cell yields for these locations was 2 600 000 cells mL^{-1}. The control algal cell yields for BDWL, SLR, and BDEL were 18%, 7.2%, and 9% of RR's yield respectively (see Figure 2.2).

Copper and nickel IC_{50}

The median copper inhibition concentration (copper IC_{50}) and the median nickel inhibitory concentration (nickel IC_{50}) of algal growth were calculated by computer software and can be found in appendix B. The one way ANOVA showed that Log_{10} copper IC_{50} value was significantly different for locations ($p = 0.001$, $n = 12$). Using a Tukey pairwise comparison probability, only the IC_{50} of BDWL water that was not significantly different ($p>0.05$) from that of BDEL water. Figure 2.3 on the top shows the copper IC_{50} value estimation with 95% confidence limits. A one way ANOVA shows that

the log 10 nickel IC_{50} was significantly different ($p = 0.04$, $n = 14$) among locations. A Tukey pairwise comparison probability calculated that the nickel IC_{50} of RR water was significantly ($p < 0.05$) different from those of the other locations, but there was no significant difference in the nickel IC_{50} among the other locations (OR, BDWL, BDEL and SLR). Figure 2.3 on the bottom shows the nickel IC_{50} value estimation with 95% confidence limits.

Figure 2.2 The control of *S. capricornutum* growth on copper (■) and nickel (□) toxicity tests for all locations. (mean ± SD, n = 60, abbreviations stand for location).

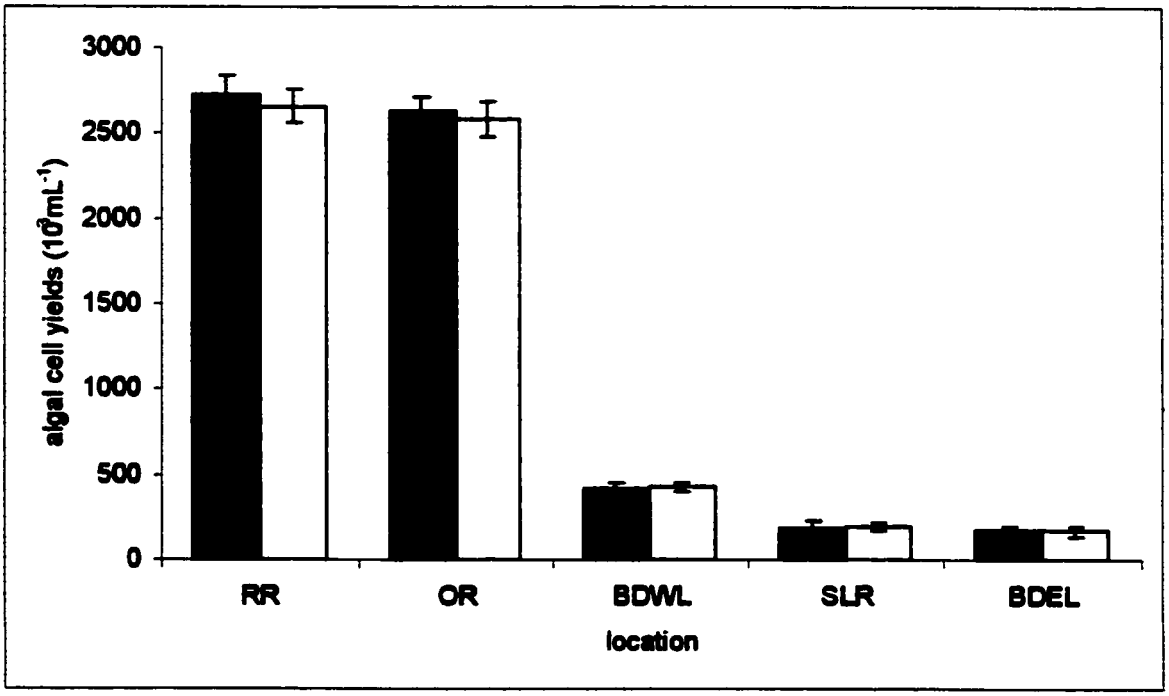
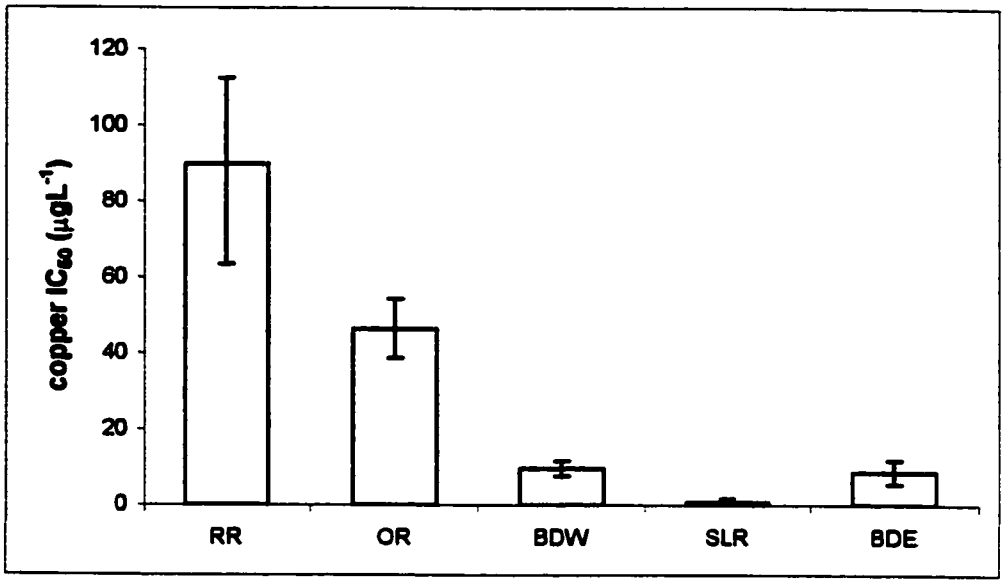

3000
2500
2000
1500
1000
500
0
algal cell yields (10^3mL^{-1})
RR
OR
BDWL
SLR
BDEL
location

Figure 2.3 The copper IC_{50} (top) and nickel IC_{50} (bottom) estimation of *S. capricormutum* for all locations. (n_{copper} =12 and n_{nickel} = 14, bars represent 95% confident limits, abbreviations stand for location)


120
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copper IC_{50} (μgL^{-1})
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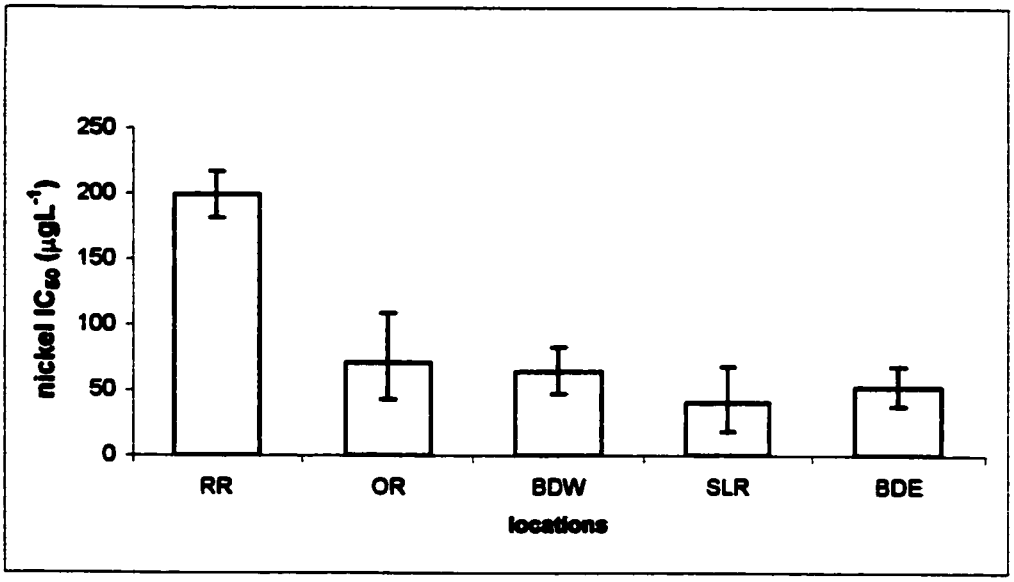
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100
50
0
nickel IC_{50} (μgL^{-1})
RR
OR
BDW
SLR
BDE
locations

2.5.2 The 48 h *Daphnia magna* tests

The median lethal concentration (LC_{50}) of copper or nickel for *Daphnia magna* is found in appendix B. Using a one way ANOVA, the LC_{50} of copper for *D. magna* was found to be significantly different for each location ($p = 0.0001$, $n = 13$). A Tukey, pairwise comparison test showed that the Cu LC_{50} of RR water was significantly different ($p < 0.05$, $n = 13$) from those of the other water samples. The Ni LC_{50} of *Daphnia magna* was significantly different ($p = 0.0001$, $n = 13$) between locations. Ni LC_{50} of RR water was the highest of all the samples. Either the copper LC_{50} or nickel LC_{50} was not significantly different for BDWL and BDEL.

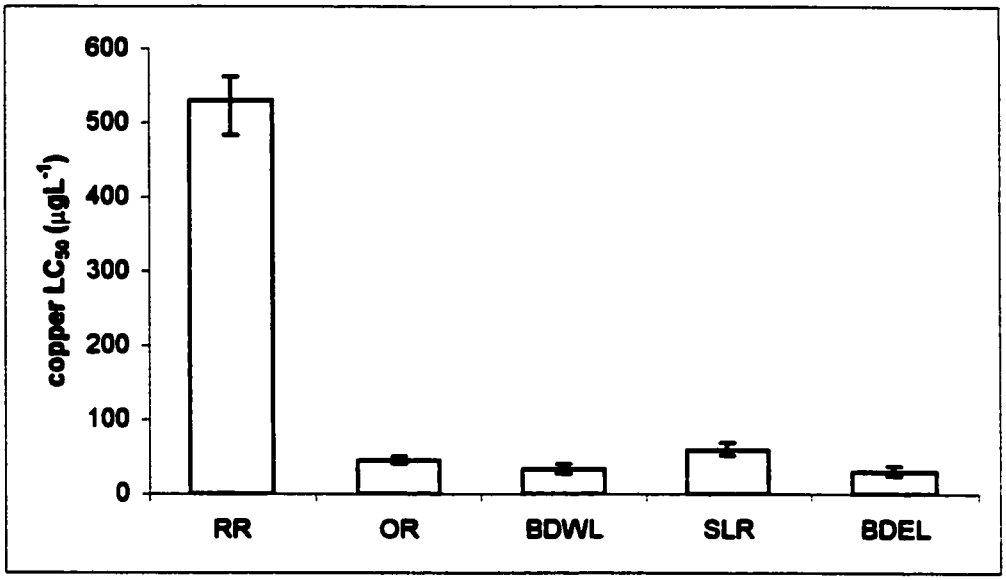
The sensitivity of organisms.

There was a significant difference ($p = 0.0001$, $n = 60$) in the sensitivity of organisms S. *capricornutum* was more sensitive than *D. magna* for both the copper toxicity test ($p = 0.024$, $n = 30$) and the nickel toxicity test ($p = 0.0002$, $n = 30$).

The toxicity of metals

There was a significant difference in the toxicity of copper and nickel. Copper is more toxic than nickel ($p = 0.0001$, $n = 60$) in both the 48 h *Daphnia magna* and the 72 h *S. capricornutum*.

Figure 2.4 The copper LC_{50} (top) and nickel LC_{50} (bottom) for *Daphnia magna* at all locations. (bars represent 95% confident limits, n = 13 abbreviations stand for the location)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copper LC_{50} (μgL^{-1})
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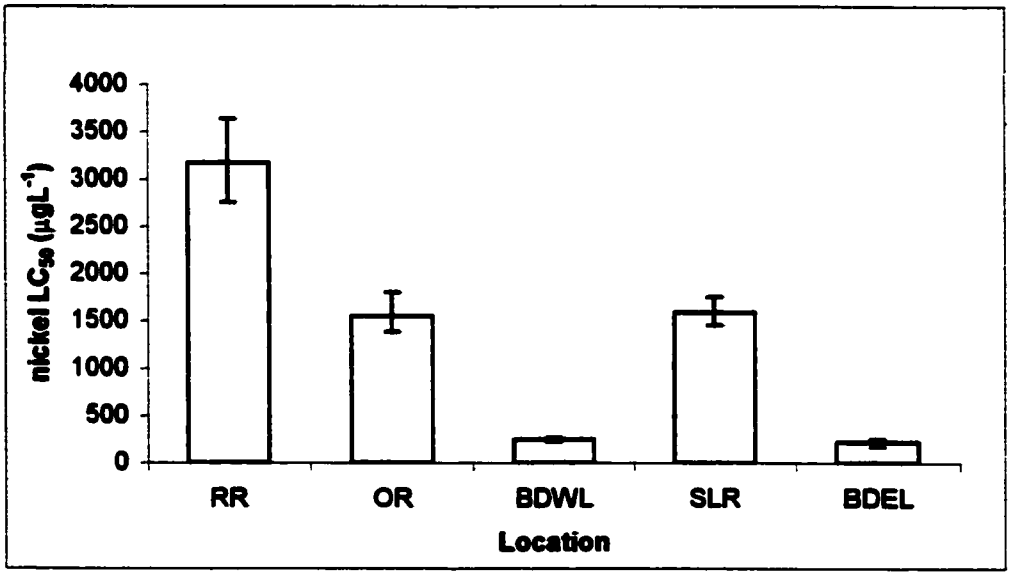
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nickel LC_{50} (μgL^{-1})
RR
OR
BDWL
SLR
BDEL
Location

2.6 Discussion

The 72 h S capricornutum test

The copper toxicity to S. capricornutum

The control sample algal cell yields (water sample plus macro and micronutrition) in RR and OR water were higher than those in the other water samples. This was probably due to the humic substances in the water samples, which either stimulated alga growth (Rashied and Prakash 1976) or protected the algae from toxic elements. Rashied and Prakash (1976) found that addition of yellow humic water to artificial seawater (ASW) or marine synthetic medium (ESWA) increased unialgal dinoflagelate growth in the culture. They suggested that the presence of humic substances was linked to the algal cell metabolisms.

The copper IC50 value for RR water (89.8 μgL^{-1}) was the highest of all the water samples. The ability of RR organic carbon to complex copper was postulated to reduce the concentration of free copper ion in the solution. This result agrees with the conclusions of Sunda and Lewis (1978), Dally et al (1990), Hollis (1996). Sunda and Lewis (1978) studied the effect of copper on the division rate of unicellular algae, *Monochrysis luthery* in media with differing concentrations of natural organic ligands. Filtered river water containing a high concentration of organic matter was added in different proportions to the culture media. There was a decrease in copper toxicity with increasing copper complexation.

The possible reason is that the high calcium concentration in the RR water also effectively competed with the free copper ion for binding sites on the membrane cell of the algae. Pagenkoff (1983) found that increasing hardness (as $CaCO_3$) reduced copper toxicity for Rainbow trout and Fathead minnows. The copper LC_{50} of rainbow trout

increased from 12.7 to 38 μgL^{-1} with an increase in hardness from 12 to 99 mgL^{-1} $CaCO_3$. The range in hardness is similar to the difference between lakes on the Canadian Shield and Lake Ontario. Winner (1986) found that the chronic copper toxicity of *D. pulex* in high DOC and hardwater was higher than that of low DOC and hardwater. This implies that the protection provided by DOC is greater than that for Ca.

The copper IC_{50} of BDWL water (9.6 μgL^{-1}) was higher, not significantly than the IC_{50} of BDEL water (8.7μgL^{-1}). There was a decrease in aromatic, aliphatic and carboxylic carbon from BDWL to BDEL (Driscoll. Pers.com). The structure of the organic carbon in BDWL water may contain more low molecular organic carbon compared to RR and OR water and low molecular organic carbon complex copper can enhance copper toxicity (Borgman and Ralph 1983, Borgmann and Charleton. 1984). Borgman and Ralph (1983) studied the effect of copper – amino acid complexation. *D. magna* was exposed to 1 and 4 mM β-alanine, 200 and 800 μM glycine, 200 μM glutamic acids and 250 and 1000 μM of Tris. It was expected that the addition of organic complexing agents would greatly increase the EC_{50}s. However, the toxicity was greater than predicted, suggesting that copper- amino acids complexes are toxic. Furthermore, Borgman and Charleton (1984) expanded the study of copper complexation. Artificial medium, artificial medium plus algae, and natural water from the Hamilton Harbour (canal) and Lake Ontario with and without Tris addition were used to determine the role of copper complexation. It found that the copper EC_{50} increase in the following order: inorganic medium, lake water, inorganic medium plus chlorella and canal water. With the addition of Tris, the copper- EC_{50} increased in the following order: lake water, canal water, inorganic medium and inorganic medium plus algae. The results suggested

that natural water complexing agents from lake water were more toxic than the copper-Tris complex. Similarly, natural complexing agents from the canal produced copper complexes, which were toxic.

The copper IC_{50} value of SLR water (0.64 μgL^{-1}) was the lowest and was significantly different from the IC_{50} for copper of the other waters. This was probably due to the high calcium levels in the SLR water competing for the organic carbon, resulting in more free copper in the solution. Winner (1986) evaluated the effect of water hardness and HA on the chronic toxicity of copper to *D. pulex*. He used reconstituted water with varying hardness and HA concentration. He demonstrated that the low concentration or absence of humic acid in hard water increased the chronic toxicity of copper to *D. pulex*. At higher concentrations of Ca^{2+} can more effectively compete for binding sites on the HA molecule with a resulting release of Cu^{2+} into the test water.

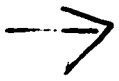
The nickel toxicity to S. capricornutum

We found that the Raisin River samples showed a significantly lower nickel toxicity when compared with the other water samples. The IC_{50} values for nickel among water samples from OR, BDWL, SLR and BDEL were not significantly different. Nickel IC_{50}s for all locations are found in appendix B. This suggests that the high level of both organic carbon and calcium protected *S. capricornutum* from nickel toxicity in the RR for the same reasons as discussed above.

Differences in nickel toxicity among water samples from OR, BDWL, BDEL and SLR were not significant. It seems that the organic carbon and calcium concentrations in those waters did not play a role in protecting against nickel toxicity. DOC of OR and

BDWL did not complex nickel well since nickel-DOC complexes are not as strong as copper –DOC complex (Mandal et al 1999). Likewise, Mandal et al (1999) studied the effect of competition between Cu and Co on the lability of Ni-organic ligand complexes in the Rideau River. It was found that only 65% of nickel was bound to the small number of strong binding sites on the organic complexants in the absence of Cu and low Co. However, in the presence of Cu, a larger portion of Ni was bound to the weak binding sites of the organic complexants, forming weak nickel complexes, which are labile.

That SLR water did not protect *S. capricornutum* from nickel toxicity is probably due to the competition between Ca and Ni resulting in Ni^{2+} forming weak Ni^{2+}-FA complexes that are labile, thus releasing free Ni^{2+} ions. Mandal et al (2000) investigated the competition of Ca(II) and Mg(II) with Ni(II) for binding by a (5.4 mmol/g) well characterized fulvic acid in a model solution at pH 8± 0.5. The result showed that the presence of high Ca and Mg in model solution has a considerable effect on the binding of Ni(II) by the FA. The concentrations of Ca(II) and Mg(II) were 4 orders of magnitude higher than that of nickel(II), allowing Ca(II) and Mg(II) to outcompete Ni(II) for sites where electrostatic interactions dominate. This resulted in Ni^{2+} forming weak Ni(II)-FA complexes that are labile, releasing free Ni^{2+} ions.



48 h Daphnia magna test

The copper toxicity to Daphnia magna

The LC_{50} of copper (530 μgL^{-1}) was the highest for RR water samples compared with the other locations. The LC_{50} of copper in RR was significantly different from that of OR, BDWL, BDEL and SLR. Copper LC_{50}s for *D. magna* among locations are found in appendix B. High DOC and calcium levels reduced Cu toxicity to *D. magna*. Higher Cu-LC_{50} for RR samples was probably due to the combined effects of copper–DOC and calcium successfully competing with copper for binding to *D. magna*'s cell. The copper-LC_{50} for SLR was slightly but not significantly higher than that of OR. It was probably due to calcium provides more protective effect against the toxicity of copper to young *D. magna*. Likewise, Winner (1985) found that copper accumulation of *D. magna* was affected by water hardness, humic acid concentration and age. Accumulated copper in newborn daphnids exposed to copper in hard water was less than for those in soft water. The addition of 0.75 mgL^{-1} HA did not significantly affect bioaccumulation of Cu by 1-day-old daphnids in soft or medium water but significantly decreased bioaccumulation in hard water. They pointed out that during the life cycle of *D. magna*, the first 7 days are characterized by energy being used for the completion of growth, none going into reproductive metabolism. After 7 days, most growth was completed and most energy was diverted into egg production. *D. magna* prefer to live in moderately hard water (Pennak 1983, Rand 1995).

The copper LC_{50} for the BDWL sample was not significantly different from that for the BDEL sample probably due to copper-organic carbon complexes being available to *D. magna* cells.

The nickel toxicity to D. magna

The LC_{50} value of total nickel for the RR sample (3168 μgL^{-1}) was significantly higher than the LC_{50}s for samples from other locations. The results demonstrate that DOC and calcium in RR water were successfully protecting *D. magna* from nickel toxicity. The nickel LC_{50} for SLR water was slightly higher than that for OR waters. Neonates of *D. magna* seemed more tolerant of the calcium in the SLR sample.

The nickel LC_{50} value for the BDWL samples was slightly higher than that for BDEL. This was probably due to the structure of organic carbon in the BDWL samples enhancing nickel toxicity (Guy and Kean 1979, Borgman and Ralp 1984).

The sensitivity of organisms.

Our results demonstrate that for both copper and nickel toxicity, *S. capricornutum* was much more sensitive than *D. magna*. In addition, *S. capricornutum* was more sensitive than *D. magna* to all the water samples. This result agrees with the previous studies (Michnowicz 1984; Lewis 1995; Nyholm and Peterson 1997).

The toxicity of metals

In general, the results indicate that the toxicity of copper is statistically more toxic than nickel to both *S. capricornutum* and *D. magna*. The toxicity of copper for RR, BDWL, SLR, and BDEL waters were 5, 3, 50, and 3-fold the nickel toxicity respectively. Geiss et al (2000) exposed the effluent with several metals (Pb, Cd, Zn, Ni, and Cu) and found that the copper IC_{50} value was 3-fold of the nickel IC_{50} of *S. capricornutum*. Macyafie et al (1994) found that the toxicity of copper was 4-fold of nickel to *Chlamydomonas reindhardtii* at pH 5.

2.7 Conclusion

The presence of both DOC and calcium in high concentrations resulted in significantly higher copper and nickel IC_{50}/LC_{50} values for algae *S. capricornutum* and *D. magna*. The organic carbon in RR samples complexes not only copper or nickel but also calcium. In fact, a part of the calcium likely binds to the cell membranes, and the other part of Ca competes for sites on the organic carbon. SLR samples resulted in the lowest copper and nickel IC_{50} for *S. capricornutum*. Calcium replaced or competed with copper or nickel ions to bind with the organic carbon, increasing the copper and nickel toxicity. That the DOC of BDWL offered less protection was probably due to its structure. In general the role of DOC was more pronounced than that of calcium to protect algae from copper and nickel toxicity. Since *Daphnia magna* prefers to live in moderately hard water (Pennak 1983, Rand 1995), the copper and nickel toxicity are higher in the low Ca samples from BDWL and BDEL. Neonates of *D. magna* were more tolerant to hardwater. This study also demonstrated that copper was more toxic than nickel, and *S. capricornutum* was a more sensitive than *D. magna*.

Chapter 3

Alteration of Dissolved Organic Carbon through UVB Exposure and Subsequent Changes in Copper or Nickel Toxicity

3.1 Introduction

The effect of ambient levels of UVB radiation on copper or nickel toxicity has been an active area of research, but the role of UVB in altering the protective influence of DOC is less well known. With anticipated stratospheric ozone depletion and expected increases in UVB, there is an urgent need for reliable information on the interactions of UVB, DOC and metal speciation.

UVB alters the dissolved organic carbon (DOC) which provides protection from sunlight in aquatic systems. The effect of UVB has been observed directly in the field (Allard et al 1993, Lindell et.al 1994, Hongve 1994) and in the laboratory by simulating sunlight (Miller and Kester 1994, Miller and Zepp 1995, Frimmel 1998). Some studies showed that the photolysis products of DOM are lower molecular weight (LMW) organic compounds (Strome and Miller 1978, Frimmel 1994, Hongve 1994, Miller 1994), and DIC (Allard et al 1993, Miller and Zepp 1995, Lindell 1996) including carbon monoxide (CO), formaldehyde, acetaldehyde, acetone, glyoxal, methylglyoxal, glyoxalate, piruvate, and carbon dioxide (CO_2). The formation of CO_2 and CO are produced by decarboxylation and decarbonilation (Lindell 1994, Miller 1994).

After exposing bog water and fulvic acid solution to natural sunlight for 6 and 12 days, Hongve (1994) found that up to 67% of its original colour disappeared but the DOC was only reduced by 32%. It was suggested that that loss in colour and DOC indicated

that the high molecular weight fraction of dissolved organic matter (DOM) was reduced and the low molecular weight fraction was increased.

Likewise, Frimmel (1998) exposed aerated and non-aerated water samples to UVB radiation. There was a small (10%) decrease in DOC and a shift in molecular size to smaller molecules which were assimilated more rapidly. The UV toxicity to D*aphnia magna* was higher in the non aerated samples.

Allard et al. (1993) exposed fulvic acid from the surface water of Radsla, Sweden and commercial humic acid to UV irradiation. He found total organic carbon (TOC) in the humic solution was reduced 50 and 75% after 30 and 71 h exposure. Humic acid required 58h for 95% reduction, but fulvic acid took only 12 h of exposure to decrease its UV absorbance by 95%. The different time requirements for humic and fulvic acid may be due to the higher stability of humic acid. The main products of photoproduction after 6 hours irradiation were identified as low molecular weight compounds such as oxalate, formate, succinate and acetate with CO_2 formed in the final degradation.

Solar radiation causes significant alteration of DOM in natural waters (Miller 1994). The chemical nature of DOM and DHS has been examined using molecules that quench fluorescence (Milne et. al 1989; Green et. al 1992). Miller (1994) showed that there is a strong correlation between photochemical production of LMW carbonyl compound and the photodegradation of DOM in natural waters by looking at the absorbance of light at 300 nm. In addition, the carbon compounds produced by irradiation of natural water were formaldehyde, acetaldehyde, acetone, glyoxal, methylglyoxal, carbon monoxide, glyoxalate, pyruvate, and carbon dioxide.

Lindell et al. (1996) reported that the highest potential photodegradation of DOC into DIC and LMW carboxylic acids occured in spring/ early summer and the least photoreactive time was the fall. Photoproduction of LMW carboxylic acid are, mainly, formic and acetic acid (80%), and malonic and oxalic acid (20%). Oxygen is a limiting factor for DIC production from DOC. Humic lakes produced 8 times higher DIC than a clear lake, even though the DOC level of the humic lake was two times higher than that of the clear lake. DOC photooxidation per unit carbon must be higher in humic than clear lakes because the humic lake has a shorter residence time, and a higher input of allochthonous DOC than the clear one.

The effect of UVB radiation on the degradation of dissolved humic material (DHM) resulting in bacterial growth has been observed (Wetzel 1995, Lindell et al 1995, Lindell et al 1996, Miller and Moran 1998). They showed that low molecular weight carbon photoproducts were considered to be the compounds responsible for stimulating bacterial activity following the photodegradation of DOM.

Miller and Kester (1994) observed the photochemical effect of iron in natural seawater on the growth of phytoplankton, diatom *Skeletonema costatum.*. The results confirmed that freshly precipitated iron hydroxide in seawater can provide iron for cell growth. It suggested that iron photoproduction (Fe(II)) effectively competed with Fe(III) for cell surface ligands causing an increase in cell density, but no further increase in cell growth for a longer time occurred probably due to a steady state condition between labile iron production and loss.

It has been known that exposure to UVB radiation alters metal speciation in aquatic system, which influences the toxicity or bioavailability of the metal (see review

by Lean 1998). UVB destroys metal – organic complexes releasing more bioavailable forms. It follows that metal toxicity is due to the reduced ability of organic carbon in the environment to complex metal ions. In other words, UVB removed functional groups are important in reducing metal toxicity. Clearly the intensity and duration of the exposure can cause profound changes in DOC. Here, I used only a modest exposure to a sample of DOC fresh from a source (Raisin River) with little previous UV exposure. In addition, the sample was known to provide the highest protection from Cu or Ni toxicity. The objective of this chapter is to determine if DOC protection from copper or nickel toxicity lost when water samples were exposed to UVB radiation.

3 .2 Materials and Methods

3.2.1 Experimental procedure

Water samples were collected from Raisin River, filtered through 0.45μm membrane filters (Sartorius, cellulose acetate) and stored at 4°C in teflon bottles, wrapped with aluminium. Water samples were transferred to 50 mL quartz tubes. The tubes were placed about 15 cm under 2.65 watt/m^2 UVB light (UBL FS201T12 – UVB USA) for 5 and 10 days, then it covered by cellulose acetate film to remove shorter wavelength.

3.2.2 Optical Measurement

Absorbance (200-800 nm) of water samples was measured using Cary 100 UV-Vis spectrophotometer to estimate the photobleaching. An increase in the absorbance ratio at 250: 365 nm is due to an increase in the smaller molecular weight component of DOM (Strome and Miller 1978, Lindel 1996).

3.2.3 Carbon analyses

The DOC concentration of RR water was measured at 0 day (before exposure), and after 5 and 10 days of UVB exposure using TOC analyses – IO Analytical model 210. About 10 mL of water samples before (0 day) exposure, after 5 and 10 days exposure together with blank (0), 5, 10, 25, and 50 ppm of sodium persulfate standard were full fill in the small tubes, then put in the TOC analyser machine.

3.2.4 72 h *Selenastrum capricormutum* test

The inhibition of alga growth as an indicator of copper or nickel toxicity is the same as in the previous experiments (see chapter 2). The range of was 0.5 to 500 μg Cu L^{-1} and 10 -1000 μg Ni L^{-1}.

3.2.5 Windermere Humic Aqueous Model –Waters (WHAM)

WHAM is a computer program used to predict copper and nickel speciation and its concentrations in the water samples before (0 days) and after 5 and 10 days UVB exposure.

3.3 Statistical Analyses

The cell yield of the control for copper and nickel were compared by using a nonparametric Mann-Whitney-U test (Zar 1999). Alga growth at each concentration of copper and nickel were calculated and the IC_{50} was calculated. IC_{50} estimates values and 95% confidence limits were calculated by probit analyses (LC_{50} calculation software program, Harras et al.1986). Comparison of IC_{50} and their confidence limits among time exposure were analysed with a non parametric Kruskal Wallis (Systat® 7.01. software package for windows).

3.4 Results

3.4.1 Photobleaching

Figure 3.1 shows the effect of UVB exposure on the bleaching of the RR sample. The mean (±SD) absorbance ratio 250: 365 nm was 6.8 (±0.14) before exposure, 9.33 (±0.36) at 5 days UVB exposure and 10.33(±0.20) at 10 days UVB exposure. The absorbance ratio was reduced by 37% after 5 days UVB exposure and by 52% after 10 days UVB exposure (See table 3.1).

Table 3.1 Absorbance of triplicate RR sample at 0 (before exposure), 5 days, and 10 days exposure to UVB (n=3).

before exposure		5 days expose to UVB		10 days expose to UVB	
250 nm	365 nm	250 nm	365 nm	250 nm	365 nm
0.8950	0.1290	0.4162	0.0435	0.2042	0.0020
0.8998	0.1351	0.4265	0.0448	0.3678	0.0361
0.8871	0.1300	0.4110	0.0461	0.3604	0.0344

3.4.2 DOC level

Figure 3.2 shows the relationship of the absorbance at 265 nm to the DOC level of RR samples by the time of UVB exposure ($R^2 = 0.977$). The DOC level was reduced by only 13% and 20% at 5 days and 10 days exposure respectively (See table 3.2).

Table 3.2 DOC concentration (mgL^{-1}) and absorbance (265 nm) of RR sample at 0 (before exposure), 5, and 10 days exposure to UVB

	0 day	5 days	10 days
DOC (mgL^{-1})	24.4	21.2	19.6
A(265) nm	0.7676	0.32695	0.2890

Figure 3.1 The absorbance of Raisin River sample at 0, 5 and 10 days exposure to UVB.

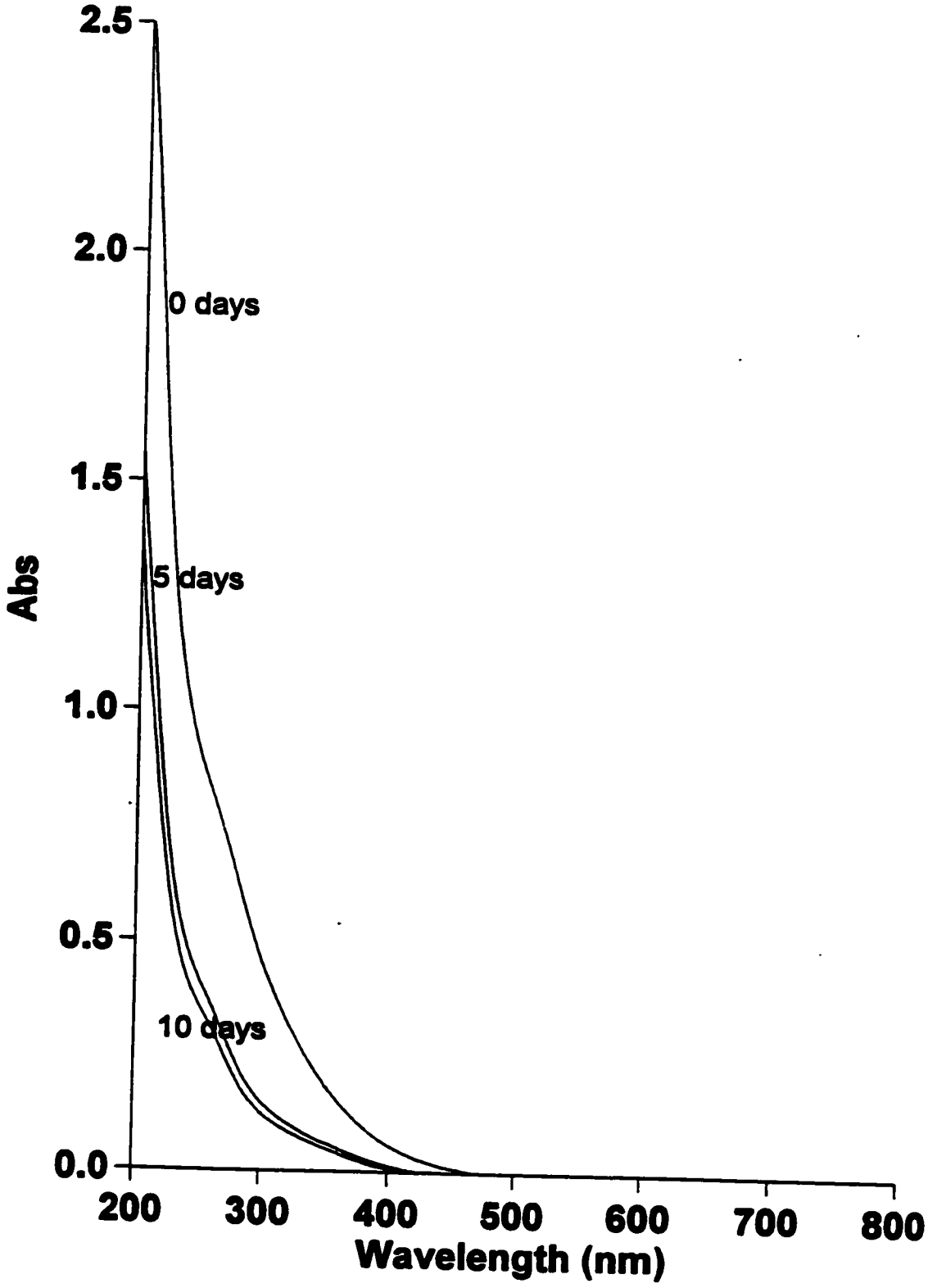
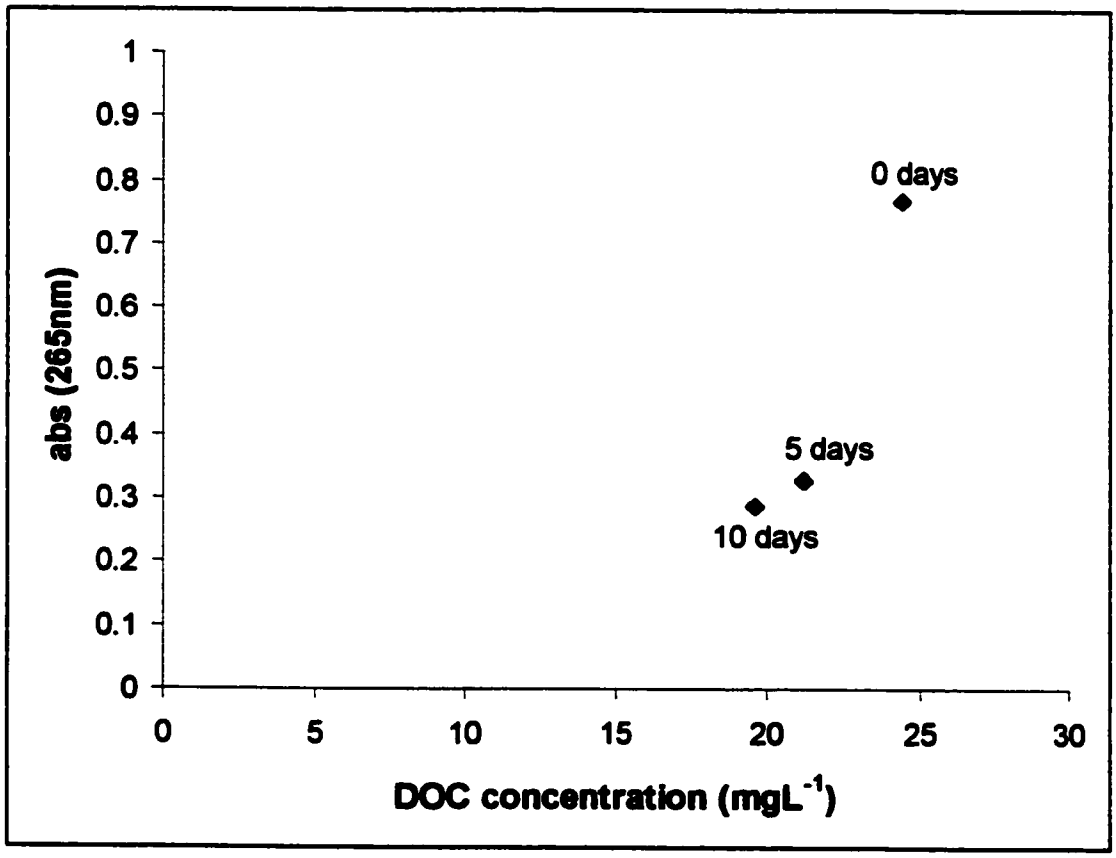

2.5
2.0
1.5
1.0
0.5
0.0
Abs
0 days
5 days
10 days
200
300
400
500
600
700
800
Wavelength (nm)

Figure 3.2 Plot of the absorbance at 265 nm to DOC level of RR samples by increased UVB exposure.


1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
abs (265nm)
0 days
5 days
10 days
0
5
10
15
20
25
30
DOC concentration (mgL-1)

3.4.3 Alga growth inhibition.

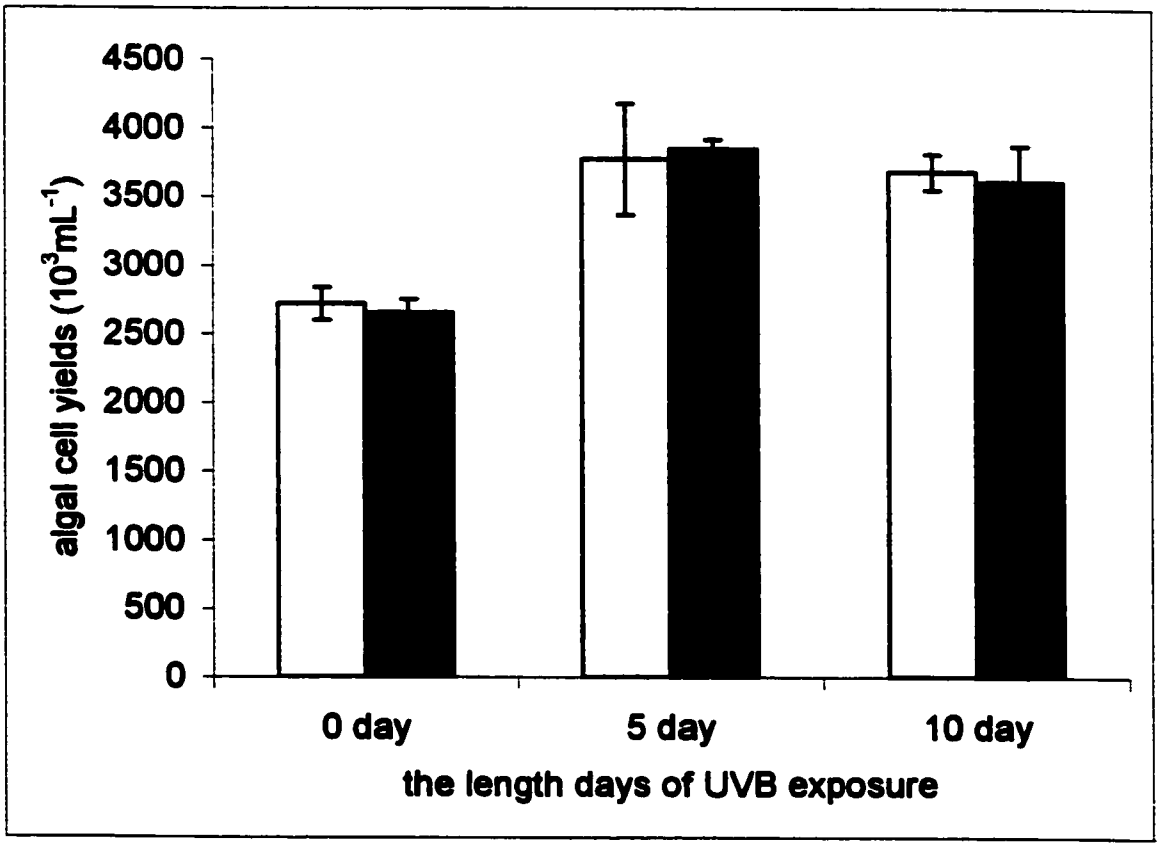
Control Standard

Alga grown in milli-Q water spiked with nutrient (without EDTA) was used as a control standard for the copper and nickel toxicity tests. The mean (± SD) of alga cell in this standard control was 180 (±22).10^3 for the copper test and 173 (±25).10^3 for the nickel test (See appendix C).

Control sample of alga growth

The RR sample exposure to UVB radiation for 0, 5, and 10 days spiked with nutrient (without EDTA and copper or nickel) was used as a control growth of algae. The control of alga growth was the same for both copper and nickel toxicity tests with UVB exposure (Figure 3.3). The control algal cell yield increased by 37% at 5 days UVB exposure and 36% at 10 days UVB exposure.

Figure 3.3 Control of alga growth of *S. capricornutum* on copper (□) and nickel (■) toxicity. (mean ± SD, n= 60)


4500
4000
3500
3000
2500
2000
1500
1000
500
0
algal cell yields (10^3mL^{-1})
0 day
5 day
10 day
the length days of UVB exposure

Copper and nickel toxicity test

Comparison of the alga growth inhibition curves with Cu or Ni additions as a function of UVB exposure can be seen figure 3.4 and appendix C for data. The sample exposed to UVB for 10 days with 1.25 μg copper inhibited 8.2% of the algal growth, while at 5 and 0 days exposure the no inhibition was observed until a level of 5 μgL^{-1}. While the initial inhibition at 5 days exposure (1.6%) was lower than that at 0 days exposure (7.07%), increasing the spiked copper concentration caused a greater inhibition at 0 days exposure. Upon addition of more copper, the inhibition was greater for 10, 5 and 0 days exposure to UVB, respectively.

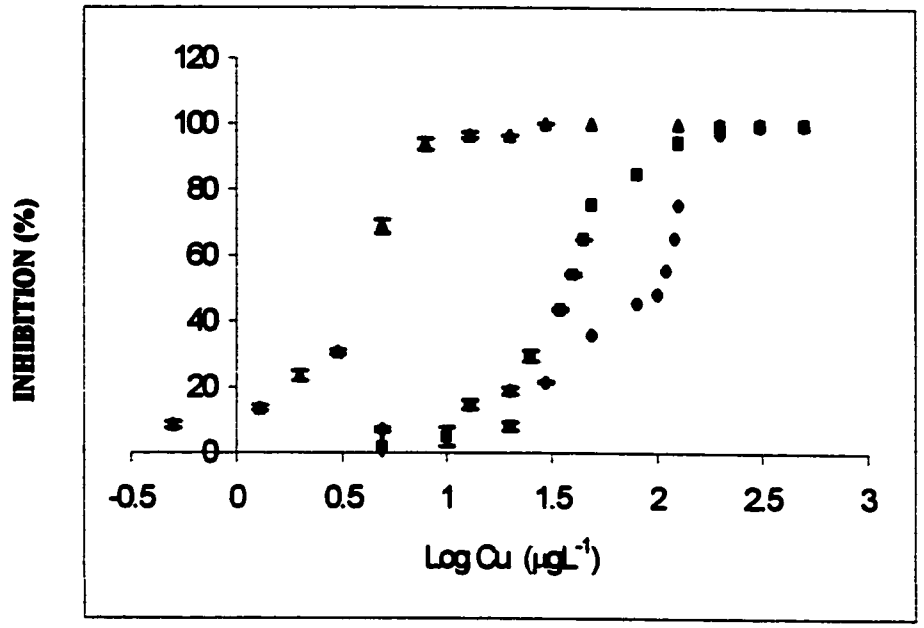
At 10 μgL^{-1} nickel, the growth of *S. capricornutum* was inhibited 27%, 22%, and 14% at 10, 5, and 0 days exposure to UVB. Additional nickel elevated the alga inhibition for all treatments.

Copper or nickel IC_{50}'s were calculated from the data in appendix C using an LC_{50} program (Harras 1988). Using a one way ANOVA, UVB exposure significantly reduced the copper IC50 ($p=0.001$, $n=9$). Using a Tukey pairwise multiple contrasts, the copper IC_{50} was significantly different between 0 and 5 days exposure ($p = 0.012$, $n= 9$), 0 and 10 days exposure ($p= 0.01$, $n=9$), but it was not significantly different between 5 and 10 days exposure (see appendix D). The IC_{50} (95% upper- lower confidence limits) of copper at 0, 5, and 10 days UVB exposure were found in appendix B. UVB exposure significantly increased the toxicity of nickel ($p = 0.002$, $n= 9$). Using a Tukey pairwise mean differences, only Ni IC_{50} for 5 and 10 days exposure was not significantly difference ($p = 0.415$, $n=9$). The IC_{50} (95% upper and lower confidence limits) of nickel at 0, 5 and 10 days were found in appendix B.

WHAM result

By using WHAM, copper and nickel speciation in the water samples were predicted. At the same concentration of Cu (80 μgL^{-1}), free copper ions increased 0.6% and 0.9% for 5 and 10 days UVB exposure respectively. 6 and 9% for increased UVB exposure reduced copper – organic complexes. Likewise, at 100 μgL^{-1} Ni, free nickel ions increased and nickel-organic complexes decreased by 4.1 and 6.8% respectively. At the copper IC_{50}, free copper ions increased and copper-organic complexes decreased by 0.4 and 0.7% after 5 and 10 days UVB exposure. At the nickel IC_{50}, exposure to UVB resulted in increased the free nickel ions and decreased nickel-organic complexes by 3.8 and 6.5% respectively. (See appendix F)

Figure 3.4 The inhibition alga growth (± SD) of Raisin River at 0 (◊), 5 (■), and 10 (Δ) days of UVB exposure as a function of log Cu concentration (top) and log Ni concentration (bottom).


INHIBITION (%)
120
100
80
60
40
20
0
-0.5
0
0.5
1
1.5
2
2.5
3
Log Cu (µgL-1)

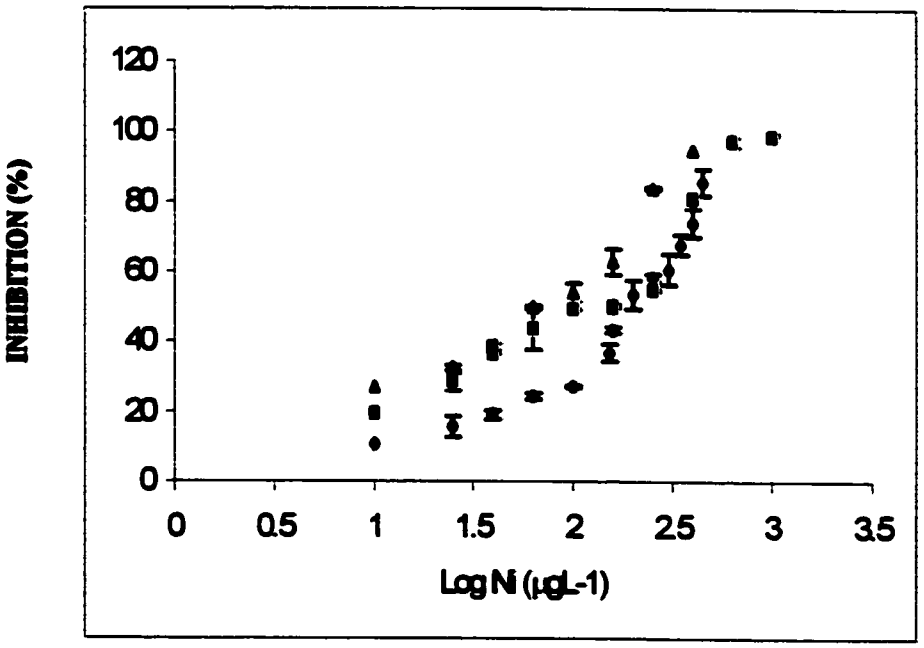
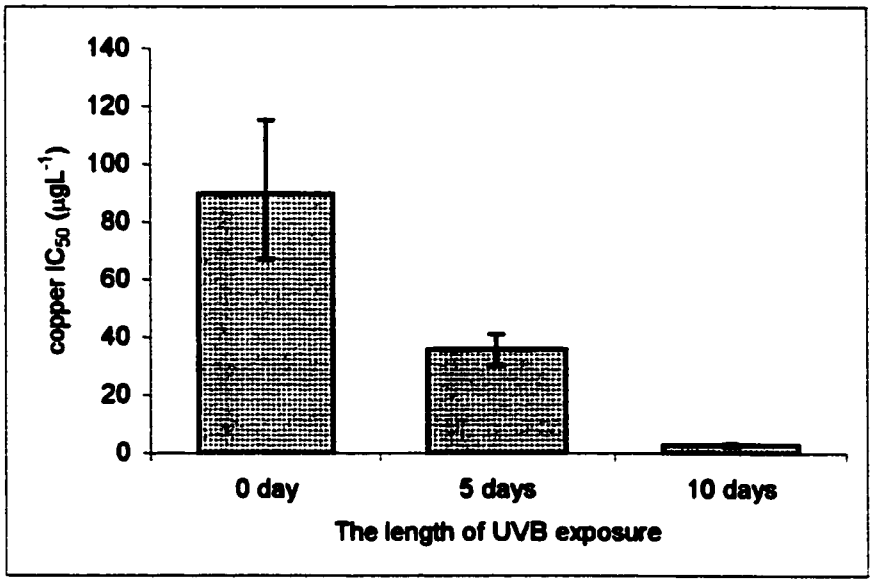
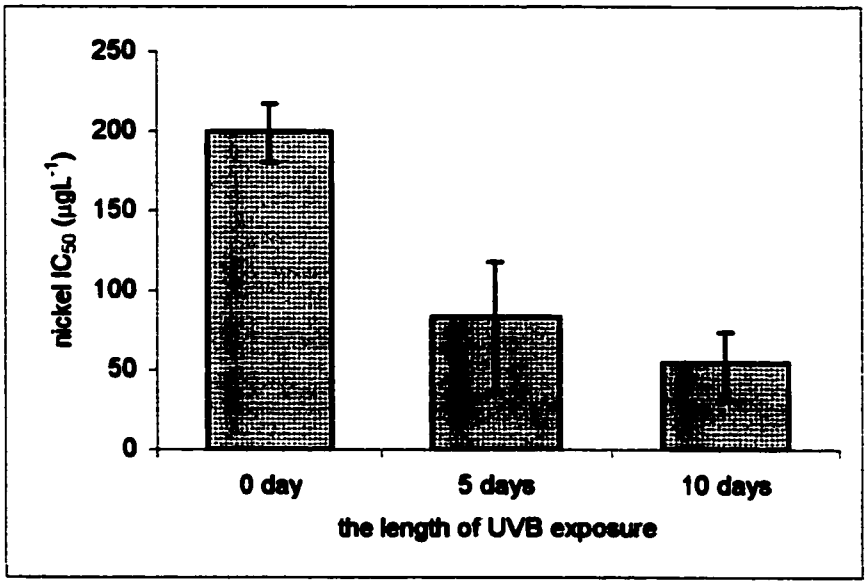

INHIBITION (%)
120
100
80
60
40
20
0
0
0.5
1
1.5
2
2.5
3
3.5
Log Ni (µgL-1)

Figure 3.5 Copper IC_{50} (top) and nickel IC_{50} (bottom) of Raisin River sample with increased exposure to UVB ($IC_{50} \pm 95\%$ CL, n = 9)


140
120
100
80
60
40
20
0
copper IC_{50} (μgL^{-1})
0 day
5 days
10 days
The length of UVB exposure


250
200
150
100
50
0
nickel IC_{50} (μgL^{-1})
0 day
5 days
10 days
the length of UVB exposure

3.5 Discussion

UVB radiation resulted in a bleaching effect in the RR sample and a small change in DOC of 13% and 20% at 5 and 10 days respectively. The increase in the ratio of 250: 365 nm indicated a decrease in molecular size (Strome and Miller 1978, Lindell et. al 1994).

The increase of the control algal cell yield after 5 days exposure may be due to an increase in iron availability for support of cellular growth by UVB exposure (the iron concentration in RR sample is 987 μgL^{-1}, see appendix A) but many other explanations are possible. No further increase after 10 days UVB exposure suggests that photochemical reactions do not further contribute to increased growth. This result agrees with Miller and Kester (1994) who irradiated the marine diatom *S. costatum*.

The increase in toxicity of copper at 5 days exposure and further increase in toxicity after 10 days exposure may be due to several reasons. First, UVB may destroy the functional groups responsible for binding Cu and Ni. This would reduce the ability of fulvic or humic acids to complex the copper or nickel, thereby increasing the amount of metal ion in the water sample, and increasing the copper or nickel toxicity. It concords with the WHAM prediction that an increased UVB exposure resulted in an increase in metal ions (from 1.9% up to 2.8% for Cu, and 74.5% up to 81.3% for Ni) and a decrease in metal-organic complexes (98 to 97.1% for copper-organic complexes and 25 % to 18.3% for nickel-organic complexes) in the solution. (see table 3.3)

Tabel 3.3 WHAM outputrelated to the algal inhibition growth

DOC (mgL^{-1})	Total metal Concentration (M) (μg)	Free metal ions (%)	Metal-organic complexes (%) DFA + FA	Inhibition (%)	The length of UVB exposure (days)	Metal
2.44 E-2	5.56E-7	2	98	50	0	*Copper*
2.44E-2	4.95E-7 (80)	1.9	98	45.5	0	*Copper*
2.12E-2	2.22E-7	2.4	97.6	50	5	*Copper*
2.12E-2	4.95E-7 (80)	2.5	97.5	84.95	5	*Copper*
1.96E-2	1.7E-8	2.7	97.3	50	10	*Copper*
1.96E-2	4.95E-7 (80)	2.8	97.1	100	10	*Copper*
2.44 E-2	1.28E-6	74.7	0.5 + 24.8	50	0	*Nickel*
2.44E-2	6.45E-7 (100)	74.5	0.5 + 25	36.7	0	*Nickel*
2.12E-2	5.38E-7	78.5	0.5 + 21	50	5	*Nickel*
2.12E-2	6.45E-7 (100)	78.6	0.5 + 21	49.01	5	*Nickel*
1.96E-2	3.5E-7	81.2	0.4 + 18.4	50	10	*Nickel*
1.96E-2	6.45E-7 (100)	81.3	0.4 + 18.3	53.9	10	*Nickel*

Second, the increase in low molecular weight DOC induced the formation of copper or nickel-low molecular weight complexes that might be available to the alga cells. Studies (Guy and Kean 1979, Borgman and Ralp 1984; Borgman and Carlton 1983; and Giesy 1983) have suggested that low molecular weight complexed organic-metal species are toxic to organisms. Uptake of these complexes may inhibit the division of cells or the alga growth. Longer UVB exposure resulted in more low molecular weight DOC possibly creating small organic metal complexes that are available to the alga cells. The loss of larger DOC may have resulted in a reduction of complexation, increasing the concentration of available metal ions, resulting in increased copper or nickel toxicity.

3.6 Conclusion

Currently researchers are attempting to relate metal toxicity to DOC levels. Here, I clearly show that the metal binding capacity of DOC is dramatically altered by modest

UVB exposure, while the total DOC was only reduced by 20%, the IC_{50} for Cu and Ni was reduced from 89.8 to 2 μgL^{-1} and 199 to 54 μgL^{-1}.

Future work should focus on more reliable identification of the functional groups altered by UVB exposure. It is known that exposure of DOC result in CO (Jones) and CO_2 (Miller 1994, Kieler and Lindell 1994) from decarboxylation and decarbonylation. The lost of these binding sites is the likely explanation for increased toxicity. There are also important environmental management implications. Currently, regulators are trying to consider ways to establish Cu and Ni loading related to water DOC levels. This may be unwise since with exposure to UVB the protective influence of DOC is lost.

Chapter 4

The Role of EDTA on the Toxicity of Copper and Nickel to *Selenastrum capricornutum.*

4.1 Introduction

The effect of ethylenediaminetetracetic acid (EDTA) on algal growth rates in media culture has been debated for many years (Lewis 1995). The use of EDTA in metal toxicity experiments is controversial, since the outcome of algal toxicity tests can be substantially altered using EDTA. Environment Canada (1991) and the American Society Testing and Materials (ASTM 1990a) recommend the use of EDTA for algae toxicity tests for several reasons. EDTA is a chelating agent that keeps iron and trace metals in solution (Nyholm and Peterson.1997). It was thought that EDTA would increase the availability of iron (Hubert and Shy 1992), and thereby promote the optimal growth of the algae (Lewis 1995). Consistency among replicates was also improved (Hughes 1991).

U.S.EPA (1994), however, recommends not adding EDTA since its potential to chelate metals influences metal toxicity. Coilie et al. (1983), Metaxas and Lewis (1991) omitted EDTA in their metal toxicity test. Nyholm and Peterson (1995) suggested using EDTA as a culturing medium only and making up the final test medium with no EDTA, iron or trace metals.

The effect of EDTA on metal toxicity depends on its concentration (Guy and Kean. 1979, Noor and Cheng 1986; Tubbing et al 1992, Huebert and Shay 1992). Noor and Cheng (1986) found that an absence of EDTA resulted in 95% of Cu being absorbed by *Eichornia crassipens.* The effect of EDTA in reducing copper toxicity was also

observed by Tubbing et al (1992). The observed toxic effect of 1, 5 and 10 μM copper increased at low EDTA. The same phenomena was observed by Guy and Kean (1979), who found that 6.62 μM EDTA became algicidal at a 6.25 μM total copper concentration, but the other EDTA concentrations tested reduced copper toxicity. Huebert and Shay (1992) observed that the absence of EDTA in a nutrient medium decreased in the toxicity of cadmium to *L. trisulca*. Geis et al. (2000) observed that the addition of EDTA as following the Environment Canada recommendations had no chelating effect on the toxicity of nickel but reduced the toxicity of Pb, Cd, Zn, and Cu.

Natural organic substrates have been known to chelate metals (Sunda and Lewis 1978, Garvey et al 1991), affecting metal speciation, and thereby, reducing metal toxicity (Winner 1985). A stimulating effect of humic substances on phytoplankton production in the sea has been observed (Prakash and Rashied 1976).

In most studies, synthetic water was used to observe the effect of additional EDTA on metal toxicity. However, little is known about the effect of EDTA on metal toxicity using natural water, which contains differing natural organic concentrations. The objective of this study was to determine the effect of EDTA for the green alga, *S. capricornutum*, in copper and nickel toxicity experiments with natural water plus additions of macro and micronutrients to ensure that the samples were not nutrient deficient. Here, the effect of addition of EDTA on the inhibition of the growth rate of *S. capricornutum* is evaluated.

4.2 Study area

The water samples were collected from five different locations with different dissolved organic carbon (DOC) and calcium: Raisin River (RR), Ottawa River (OR), Big Dam West Lake (BDWL), St. Lawrence River (SLR), and BDEL (Big Dam East Lake) (See in Chapter Two).

4.3 Materials and Methods

Experimental Waters and Chemicals

Water samples were filtered with 0.45μm filters (Sartorius, cellulose acetate) and stored at 4°C in teflon bottles, wrapped with aluminum foil until the start of experiments. The pH of water samples were measured (307 Corning pH/temperature) before exposure. To eliminate possible osmotic stress on the organisms, BDWL and BDEL, Kejimkujik waters samples were adjusted to 6.5-7 (Joubert, G. 1983) with addition of 0.1 mmol/L NaOH before experiments (Pentinen et.al.1998).

The stock solution of copper and nickel were prepared from their sulfate salts ($CuSO_4.5H_20$; 98%-102% chemical purity, BDH and $NiSO_4.6H_2O$; 98-102% chemical purity, ACS). In order to reach chemical equilibrium, test concentrations were prepared by adding stock solution to experimental waters approximately 20 to 24 hr before being exposed to the organisms.

Experimental Design

Selenastrum capricornutum toxicity test was modified from the established methods recommended by Environment Canada (Report EPS1/RM/25 1992).

96- well microplates were used to incubate Selenastrum cells spiked with different copper or nickel concentrations. Water samples were spiked with varying copper or nickel concentrations (Report EPS 1/RM/11 1990) 24 h prior to experiments. By adding aliquots into each well of microplates. Additional concentrations of copper and nickel were used in between the concentration where differences in cell yield were near the IC50 value.

Each well contained 200 µL of test solution (samples spiked with copper or nickel), 10 µL of enriched-medium, and 10 µL of fourth-day aged alga inoculum. Two microplates were used for each water sample, where one was with 300 μgL^{-1} Na_2EDTA into the enriched medium as recommended by Environment Canada (Report EPS1/RM/25 1992), and the other one was without the addition of Na_2EDTA.

The microplates were removed from their individual packaging just before use. The lids were placed on the microplates, and each was sealed with clear plastic. The microplates were incubated at a room temperature (22°C), under continuous cool white fluorescent light with an intensity of 4 klux in 100-rpm shaker for 72 hr (Gyrotori Skaker Model G2). Each concentration was replicated 4 times. Natural water unspiked as placed horizontally in ten wells of the microplates (row E of microplates) as a control for the variability in alga growth for each concentration. Milli-Q water plus nutrients unspiked copper or nickel as a standard control was placed in ten wells of the row D of microplates.

4.4 Statistical analyses

The cell yield of control from the same sample was statistically compared by using a nonparametric Mann-Whitney-U test (Zar 1999). Comparison of the control cell yields between the addition and omission of EDTA in the enriched medium was calculated by student t-test. The alga growth at each concentration of copper and nickel were calculated to get the IC_{50}. IC_{50} estimates values and 95% confidence limits were calculated with probit analyses by LC_{50} software program (Harras et al.1986). Comparison of LC_{50} and their confidence limits among location was analysed with non-parametric two-way factorial design, then continue with a Kruskal Wallis– One way anova test for multiple comparison (Systat® 7.01. software package for windows)

4.5 Results

Control Standard

Control standard of toxicity tests without addition of EDTA was 180 000 cells mL^{-1}. Control standard of toxicity test with addition of EDTA was 938 000 cells mL^{-1}

The Control sample of algal growth

Control experiments without copper and nickel additions illustrate the significant role of EDTA.The copper and nickel controls provide independent experiments for further replication.

The highest algae cell yields without nickel or copper additions and without the addition of EDTA was highest for RR and OR. The algal cell yields for BDWL, BDEL and SLR were only 18%, 7.2%, and 9% of RR's respectively.

The addition of EDTA in both copper and nickel toxicity test induced algal cell yield for BDE, BDW, and SLR. The control values for the nickel toxicity tests were similar to the control values for the copper toxicity tests. The control growth of copper and nickel toxicity for all locations can be seen in figure 4.1 and 4.2.

Figure 4.1 The control of alga *S. capricornutum* growth on copper toxicity tests for locations in the presence (■) and absence (□) of EDTA. (mean± SD, n=100).

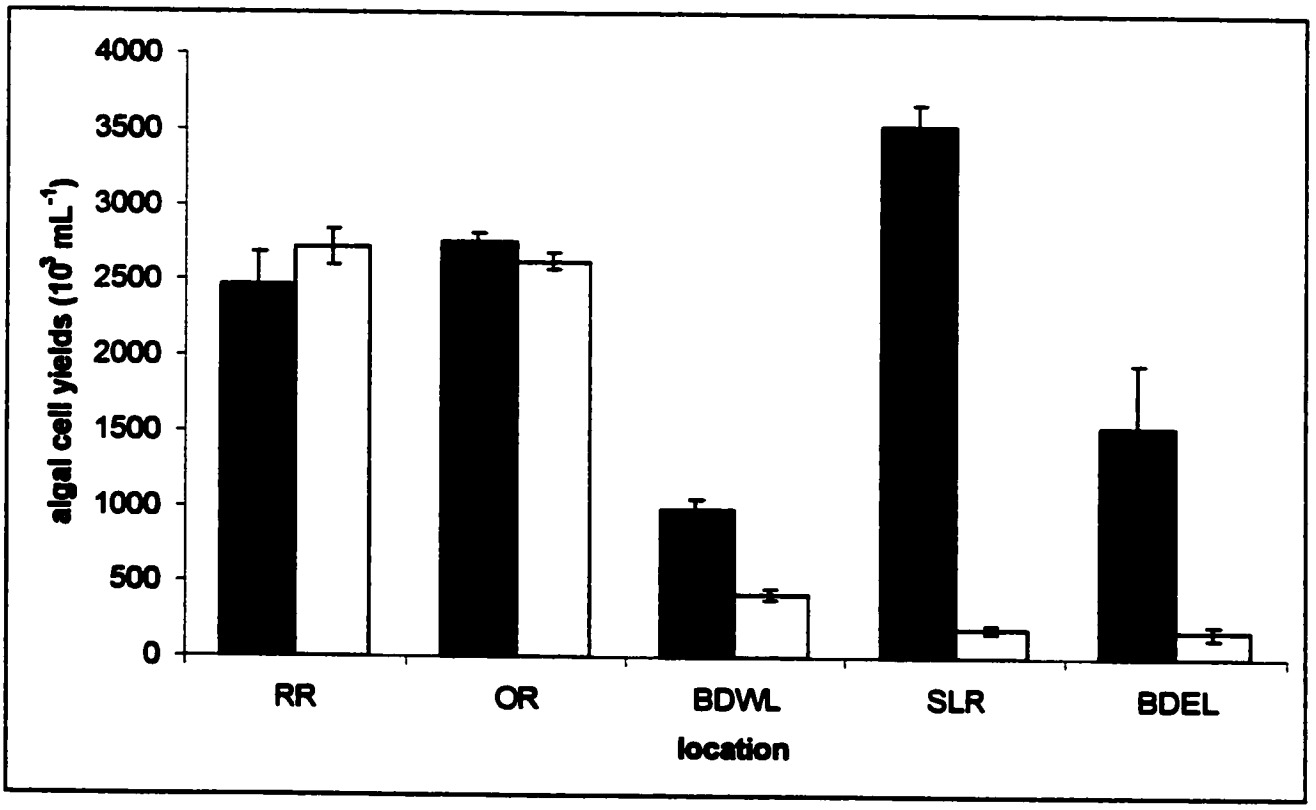
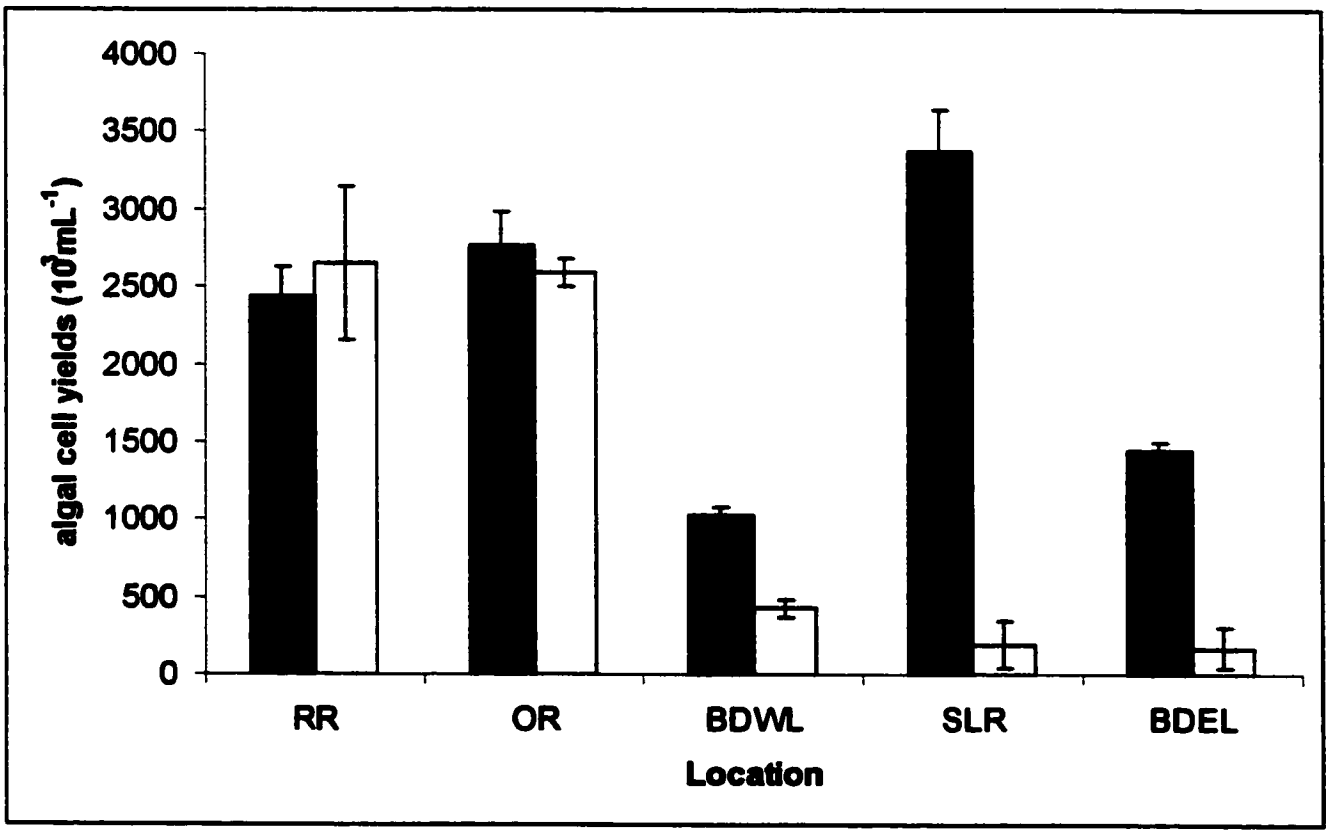

4000
3500
3000
2500
2000
1500
1000
500
0
algal cell yields (10^3 mL^{-1})
RR
OR
BDWL
SLR
BDEL
location

Figure 4.2 The control of *S. capricornutum* growth on nickel toxicity for all locations in the presence (■) and absence (□) of EDTA. (mean ± SD, n=100).

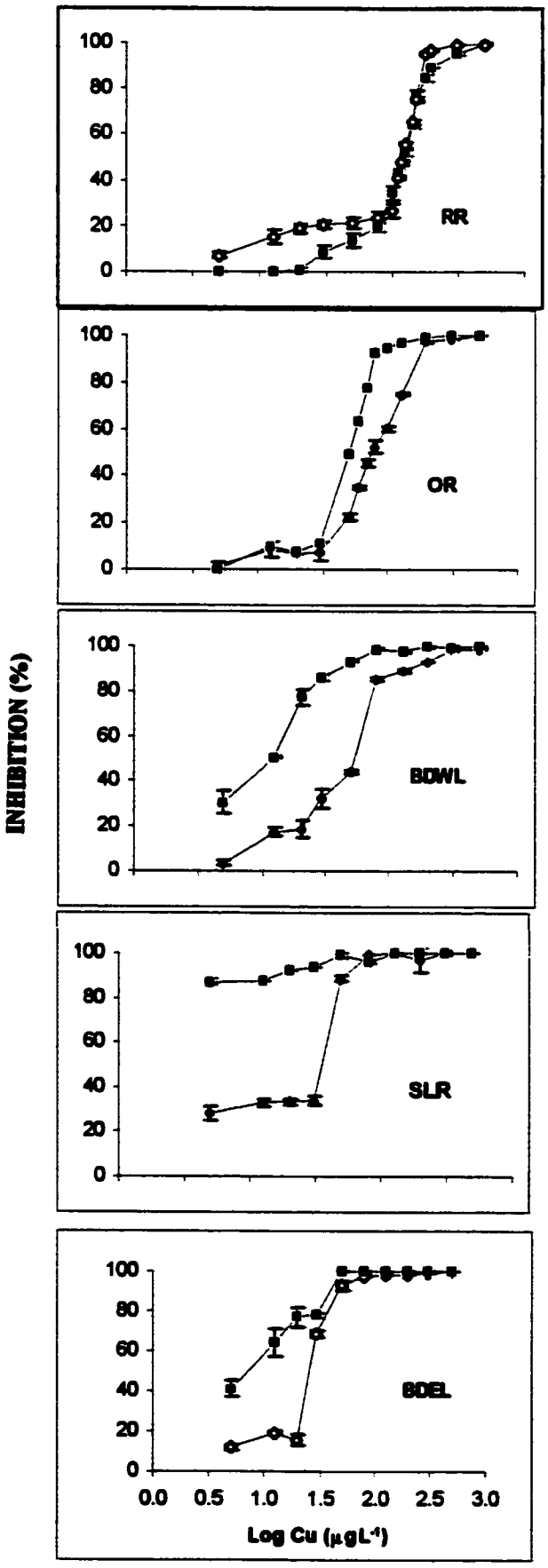

4000
3500
3000
2500
2000
1500
1000
500
0
algal cell yields (10^3mL^{-1})
RR
OR
BDWL
SLR
BDEL
Location

Copper toxicity

The comparison of inhibition of alga growth response by adding and omitting EDTA on copper toxicity can be seen in figure 4.3. Without EDTA, 5 μgL^{-1} Cu inhibit alga growth by about 7% in RR water, but at the same concentration with addition of EDTA, the inhibition was not observed. The inhibition of alga growth started only at a concentration of 20 μgL^{-1} copper in the presence of EDTA and then increased with increasing copper concentration. With OR water, inhibition (1%) was observed at the lowest copper concentration added (5 μgL^{-1}) in the presence of EDTA, and inhibition increased with added copper. Samples without EDTA resulted in even greater inhibition at the 5 μgL^{-1} copper concentration. Omission of EDTA at 5 μgL^{-1} copper repressed about 30% of algal growth, whereas addition of EDTA at the same copper concentration decreased inhibition to about 3% with BDWL water. In other words, omission of EDTA at BDWL water enhanced the inhibition about ten-fold from EDTA addition at the same copper concentration. With no EDTA, the lowest algae cell yield was observed at SLR water. At 5 μgL^{-1} copper, alga growth was inhibited up to 86%.

Using the LC_{50} software program and the data in appendix C, the copper IC_{50} for each water sample with or without EDTA were calculated. The addition of EDTA in the enriched medium did not significantly increase copper IC_{50} for RR water but the addition of EDTA in the enriched medium did increase significantly ($p=0.05$) copper IC_{50} for OR, BDWL, BDEL, and SLR water. The copper IC_{50} value (confidence limits) for OR, BDWL, BDEL, and SLR water increased from 46.3 (38.6 –54.2) up to 79.8 (74.2 – 86.3) μgL^{-1}, 9.6 (7.7 - 11.6) up to 41.6 (34.5- 49.7) μgL^{-1}, 8.7 (5.5 -11.8) up to 22.8 (14.4 –33) μgL^{-1}, and 0.6 (0.1 - 1.6) up to 19.63 (9.2 – 32.6) μgL^{-1} (see appendix B)

Figure 4.3 Inhibition alga growth of *S. capricornutum* with (■) and without (◊) EDTA on copper toxicity tests for locations : Raisin River (RR), Ottawa River (OR), Big Dam West Lake (BDWL), Big Dam East Lake (BDEL), and St. Lawrence River (SLR)).

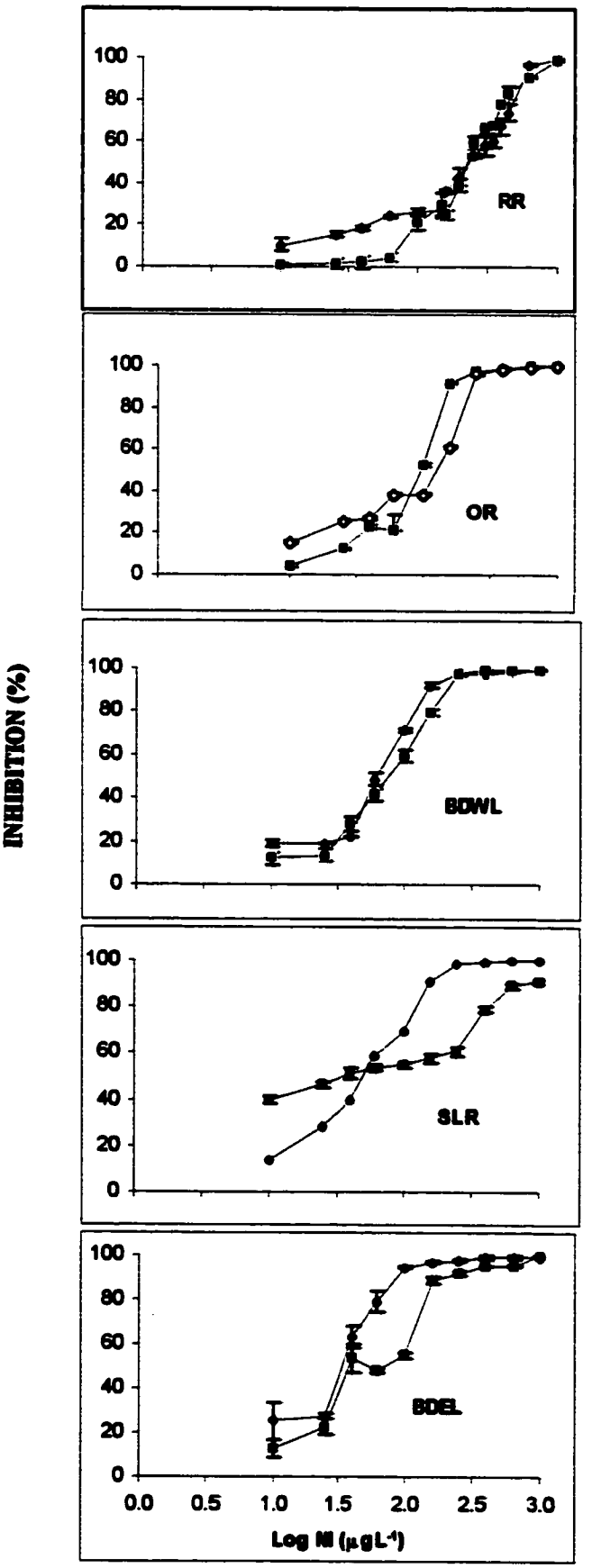

INHIBITION (%)
RR
OR
BDWL
SLR
BDEL
Log Cu (µgL⁻¹)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
20
40
60
80
100

Nickel Toxicity

The effect of EDTA on the inhibition of alga growth by nickel toxicity can be seen in Figure 4.4. With no addition of EDTA, 10 $\mu g\ L^{-1}$ nickel inhibited alga cell production by about 10% in RR water and 15% in OR water, but at the same concentration with addition of EDTA, the inhibition of alga cell growth was less than 1% in RR water and 3% in OR water. Omission of EDTA at 10 $\mu g\ L^{-1}$ nickel repressed the alga cell yield by 39% in SLR water, whereas the addition of EDTA at the same concentration was inhibited algal cell yield by only 13%. The Addition of EDTA to the nickel toxicity tests for BDWL and BDEL waters resulted in the opposite situation. Repression of alga growth by 25% and 18% was observed in the presence of EDTA at 10 μgL^{-1} nickel concentration, whereas the inhibition was only 12% and 13% in the absence of EDTA.

Using the data in appendix C, nickel IC_{50} were calculated. Using a non parametric two-way ANOVA, the nickel IC_{50} between addition of EDTA and omission of EDTA were not significantly different ($p>0.05$, $H_{EDTA} = 0.0001$, $df = 1$). EDTA slightly reduced the nickel toxicity for RR, OR, and SLR water. The nickel IC_{50} (confidence limits) for RR, OR, and SLR water increased from 199.3 (180-217) up to 225.5 (210 - 242) μgL^{-1}, 71.1 (42.5 – 109.5) up to 75.7 (57 - 98.7) μgL^{-1}, and 40.6 (18 – 68.3) up to 44.8 (36.7 – 53.6) μgL^{-1} respectively. The addition of EDTA slightly increased nickel toxicity at BDWL and significantly increased nickel toxicity for BDEL.

Figure 4.3 Inhibition alga growth of *S. capricornutum* with (■) and without (◊) EDTA on nickel toxicity tests for locations : Raisin River (RR), Ottawa River (OR), Big Dam West Lake (BDWL), Big Dam East Lake (BDEL), and St. Lawrence River (SLR)).


INHIBITION (%)
100
80
60
40
20
0
RR
OR
BDWL
SLR
BDEL
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Log Ni (µgL-1)

Discussion

Control growth

The high yields in both RR and OR on the control of alga growth was probably due to the high level of humic substances which may either stimulated the production of phytoplankton (Prakash and Rashid 1976) or protected the phytoplankton from other toxic elements. The addition of EDTA increased algal cell yields. It agrees with the study of Thellen et al (1989) and Geiss (2000). This observation provides valuable information suggesting that SLR, BDEL and BDWL contain levels of a material, which inhibits algal growth. Either some chemical is toxic or a trace element is unavailable.

Thellen et al (1989) assesed the performance of the algal microplate assay by comparing the algal cell yields growing in Algal Assay Procedure (AAP) with EDTA and without EDTA. They found that the algal cell yields in control growth without EDTA was much lower than those with addition of EDTA, suggesting that EDTA promoted algal growth by allowing better assimilation of essential macro and micronutrients or ameliorating toxicity by complexing metals.

Copper toxicity

The copper IC_{50} for BDWL, OR, SLR, and BDEL increased with the addition of EDTA. Reducing the copper toxicity in these waters may be due to the high affinity of copper for EDTA (Metaxas and Lewis 1992) since the DOC level in these waters are not as high as at RR.

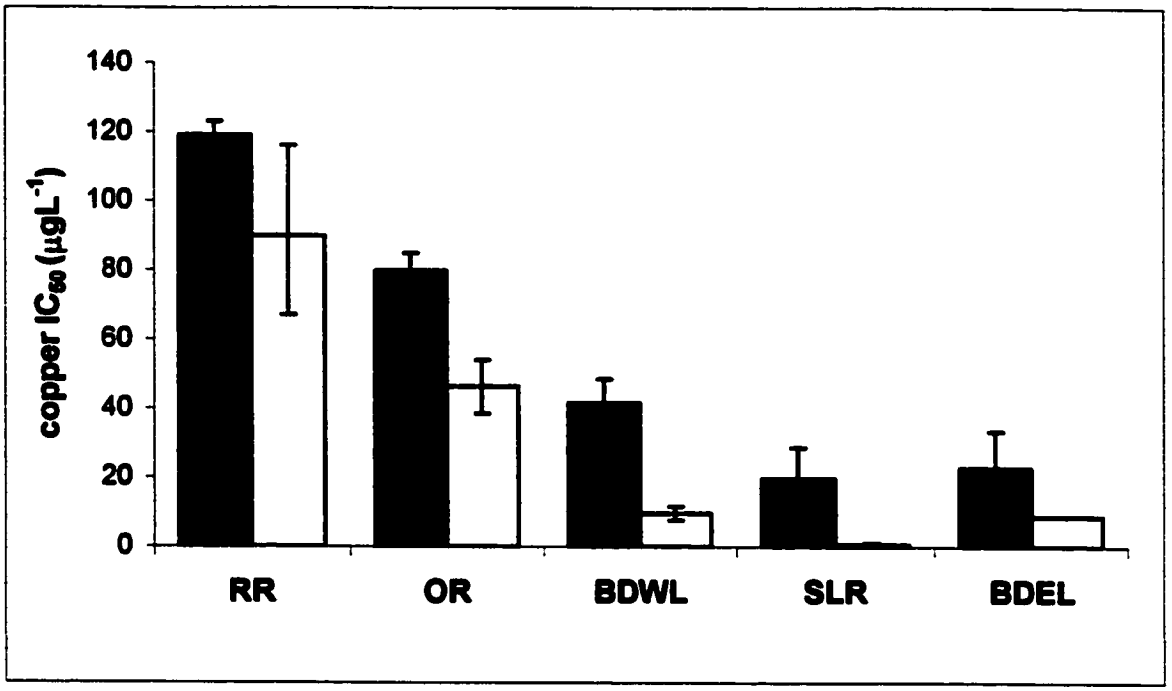
With decreasing DOC in the water samples the copper IC_{50} decreased from > 100 to < 2 μgL^{-1}. With EDTA the copper IC_{50} for the high DOC waters was not altered but

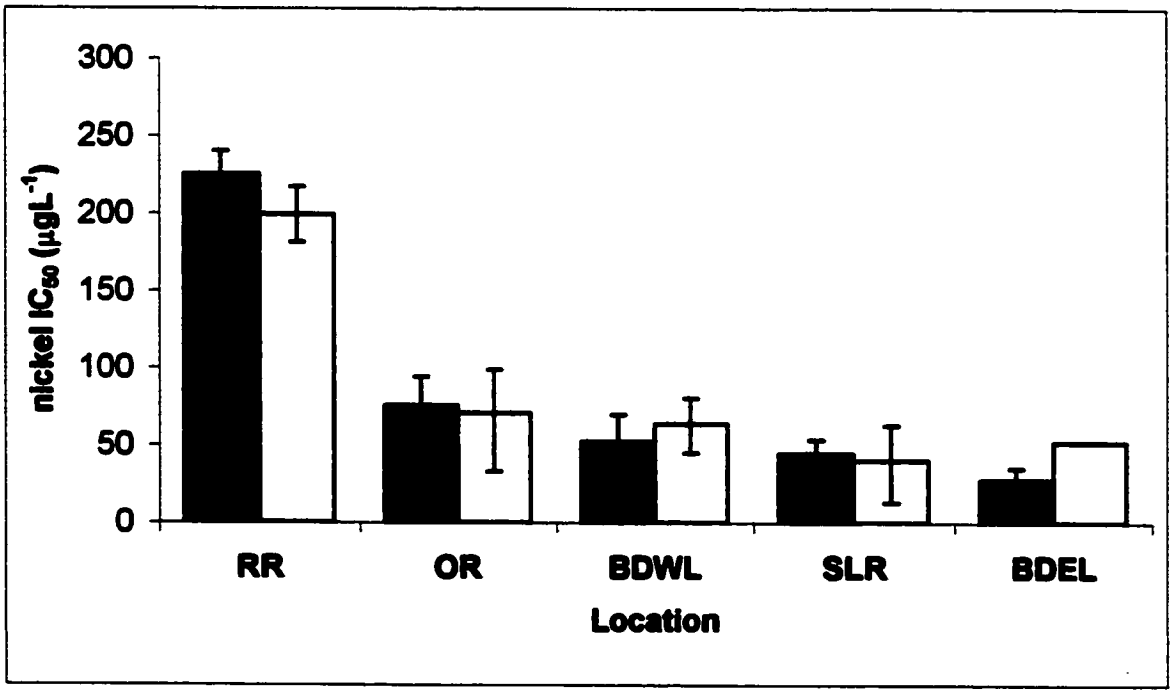
the IC50 for the low DOC waters was increased to about 20 μgL^{-1}. This illustrates that Cu^{2+} is not as toxic to *S. capricornutum* in high DOC waters as it is in low DOC waters and that EDTA can provide protection for the algae from Cu^{2+} in low DOC waters.

Nickel toxicity

Addition of EDTA did not significantly increase the nickel IC_{50} for RR, OR, SLR and BDWL water. These results are probably due to the affinity of nickel for EDTA (Zeis et al 2000). That addition of EDTA even increased the nickel toxicity for BDEL water is poorly understood. One possible explanation is because Al and Mn concentrations in these waters were quite high. Perhaps EDTA complexed the free Al and Mn ions resulting in more free nickel in the waters. In addition, BDEL lacked DOC

Figure 4.5 Copper IC_{50} (top) and nickel IC_{50} (bottom) with addition (■) and omission (□) of EDTA in all locations. (bars represents 95% confidence limits, n=30).


140
120
100
80
60
40
20
0
copper IC_{50} (μgL^{-1})
RR
OR
BDWL
SLR
BDEL


300
250
200
150
100
50
0
nickel IC_{50} (μgL^{-1})
RR
OR
BDWL
SLR
BDEL
Location

Conclusions

Low algal yields were observed in some of the control water samples with no Cu or Ni additions. This suggests that a toxic material was already inhibiting algal growth but with additions of EDTA the inhibition was not as severe.

Since copper has a high affinity for EDTA, the copper IC_{50} for water sources with low DOC such as SLR, BDEL and BDWL waters significantly increased with the addition of EDTA. However, the copper toxicity was not significantly reduced at RR water since this water has high DOC.

The addition of EDTA on nickel toxicity suggests that EDTA did not chelate nickel as well as it did copper. The nickel IC_{50} did not significantly increased for RR, OR, SLR, and BDWL after addition of EDTA. The nickel toxicity even increased in BDEL since the concentration of Al and Mn is quite high (1μM).

Chapter 5

Summary and Conclusions

Copper and nickel are metals with the potential to be toxic in aquatic environments. Such levels are often found in regions near smelting or mining operations. In Part 1 I reviewed this literature. While the interaction of calcium and DOC for metals has been studied for many years, the environmental significance is still not fully understood.

In Part 2, I conducted experiments on several water samples that provided a range of Ca and DOC. The 72-h *S. capricornutum* and the 48-h of *D. magna* tests were chosen since these methods are standardized and internationally used. Algae are considered to be the major primary producer in aquatic ecosystems and daphnids are one of the more common grazers or consumers of algae. As such, they are important components of aquatic foodwebs. Samples of water were collected from five locations differing in calcium and DOC levels: Raison River (high DOC and high Ca), Ottawa River (mid DOC and mid Ca), Big Dam West Lake (high DOC and low Ca), Big Dam East Lake (low DOC and low Ca) and the St. Lawrence River (high Ca, low DOC).

In the copper algal toxicity tests, I found that the IC_{50} of Raison River water was the highest (90 $\mu g l^{-1}$) of the five water samples by a considerable amount. The IC_{50} for BDWL samples (9.6 μ Cu gL^{-1}) were not significantly different from that for BDEL water (8.7 $\mu g L^{-1}$). The copper IC_{50} for SLR water was the lowest (0.6 μg Cu L^{-1}). These results showed that the high DOC and high calcium levels in RR protected the alga from copper or nickel toxicity but that Ca offered little protection for the SLR sample.

The complexation of copper by DOC was expected to reduce copper or nickel toxicity and the role of Ca^{2+} could either compete with the free copper or free nickel for membrane binding sites on the cell sites or replace the Cu and Ni bound to DOC, thereby enhancing copper and nickel toxicity.

The nickel toxicity test for *S. capricornutum* resulted in the IC_{50} of nickel for RR water sample of 199.3 μgL^{-1} and this was significantly higher from the IC_{50}s of the other water samples which were not significantly different from each other.

The copper or nickel acute toxicity test for *D. magna* showed again that the copper LC_{50} for RR water was the highest. The copper and nickel LC_{50} for SLR was slightly higher than that for OR water. The copper or nickel LC_{50} for BDEL water was the lowest and not significantly different from that of BDWL water. It seems that having both high DOC and high Ca increased protection against copper or nickel toxicity for *D. magna*. Since the neonates of *D. magna* are more tolerant to Ca and prefer to live in moderately hard water, the toxicity of copper or nickel was low in SLR water.

In Part 3, I showed that the protection against copper or nickel toxicity was reduced with UVB radiation. RR water was exposed to UVB radiation for 5 and 10 days. The toxicity of copper or nickel was measured as the inhibition of growth cells *of S. capricornutum*. Our results demonstrated that the toxicity of copper or nickel increased UVB exposure. Water samples were exposed to UV radiation equivalent to about a third of summer sun conditions for 5 and 10 days. After this exposure the toxicity tests were run. Photobleaching of the water sample was observed along with an increase in toxicity of copper or nickel.

Simple regressions of the Ca and DOC vs toxicity of all the water samples were inconclusive for the resistance of Raison River water to toxicity far exceeded all others. In this sample, the role of Ca vs DOC could not be separated. However, when samples were irradiated under low levels of UVB radiation, the resistance to toxicity was lost even though the DOC level was reduced by only 18%. This demonstrated that the protection from copper and nickel toxicity was not due to Ca since there was as much Ca after irradiation as before. Also it showed that the total DOC concentration was not as important as the kind of DOC. With exposure to UVB radiation the functional groups responsible for binding Cu and Ni were lost.

The role of EDTA in the toxicity of copper or nickel was investigated in part 4. We found that addition of EDTA to the RR sample did not significantly reduce copper toxicity but adding EDTA to BDWL, BDEL, and SLR water increased their IC_{50}s. This shows that EDTA complexed the free copper ions in those samples. The addition of EDTA in the nickel toxicity tests, however, did not significantly increase the Ni IC_{50} for RR, OR, BDWL and SLR water suggesting that EDTA did not chelate nickel as well as it did copper.

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APPENDIX A

Water chemistry of samples: Big dam East, Big Dam West, Raisin River, St. Lawrence River, and Ottawa River. The water chemistry data for Raisin River and St.Lawrence River is provided from the report of Atmospheric Change Impacts (1994), for Big Dam East and Big Dam West is from the Geological Survey of Canada (2000) and that of Ottawa River is from the PhD thesis of Kevin H. Telmer (1996).

Water chemistry	Big Dam East (µg/l)	Big Dam West (µg/l)	Raisin River (µg/l)	St. Lawrence River (µg/l)	Ottawa River (µgl/L)
Alkalinity	920	70			642
PH	5.9	5.0	8.	8.	7.4
Color	21	94			
Conductivity	23.8	30.1			
C total DIC (CO_2,HCO_3,CO_3^-) DOC	3700	10,500	 51,800 24,900	 22,100 3,500	10,000 l
Chloride (Cl^-)	4,060	4,840	6270	20,800	887.5
Sulphate (SO_4)	1,800	1,690	6600	25,900	4800
N_2 total NO_2-N-F (nitrite filter) NH_3-N-F NO_3NO_2-F TKN-N-F (total kjeldahl)	710	111	 8 111 24 1830	 8 13 153 265	700
Sodium (Na)	2,930	3,520	3,300	11,100	920
Potassium (K)	210	307	1,000	1,200	312
Calcium (Ca)	606	641	86,500	37,200	2380
Magnesium (Mg)	381	364	5,800	8,500	739.2
Aluminum (Al)	72	198	10	< 10	83.7
Iron (Fe)	36	165	987	6	279
Nickel			< 2	< 2	-
Copper Barium			< 1	1	- 96.1
Manganese (Mn)	13	15	1110	1	149
SRP-P-F (Soluble reactive phosphorous) TP-P-F TP-P-UF SO4			45.6 60.9 84	2.1 6.1 15.1	0.2 480

APPENDIX B

Copper IC 50 and Nickel IC50 with 95% upper and lower confidence limits.

1. *Selenastrum capricornutum*

Location	Copper IC 50 (95% below- upper CLs) (μgL^{-1})		Nickel IC50 (95% below – upper CLs) (μgL^{-1})	
	EDTA	No EDTA	EDTA	No EDTA
Raisin River (RR0D)	119 (113 – 124)	89.76 (67.08 – 115.2)	225.49 (210 – 241.5)	199.32 (180 – 217)
Raisin River (RR5D)		35.89 (30.64–41.46)		83.37 (48.85-131.64)
Raisin River (RR10D)		2.86 (2.40-3.37)		54.42 (35.02-77.53)
Ottawa River (OR)	79.87 (74.2 – 86.3)	46.28 (38.62 – 54.16)	75.69 (57.01 – 98.76)	71.09 (42.54 – 09.53)
Big Dam West Lake (BDWL)	41.55 (34.48– 49.67)	9.640 (7.671 – 11.61)	52.705 (35.26– 73.67)	63.91 (47.54–83.35)
Big Dam East Lake (BDEL)	22.75 (14.39 – 32.91)	8.65 (5.47 – 11.78)	27.53 (18.63– 36.86)	51.92 (37.64 – 68.30)
St. Lawrence River (SLR)	19.63 (9.155– 32.60)	0.64 (0.11 –1.58)	44.81 (36.68 – 53.62)	40.59 (18.00– 68.33)

2. Daphnia magna

Location	Copper LC50 (95% below- upper CLs) (μgL^{-1})	Nickel LC50 (95% below – upper CLs) (μgL^{-1})
Raisin River (RR)	530.04 (487.13 – 561.78)	3168 (2753 – 3637)
Ottawa River (OR)	45.05 (40.86 – 50.52)	1551 (1380 – 1799)
Big Dam West Lake (BDWL)	32.90 (27.45 – 39.51)	243 (223 – 266)
Big Dam East Lake (BDEL)	30.1 (24.78 – 36.62)	219 (173 – 253)
St. Lawrence River (SLR)	58.35 (50.49 – 67.88)	1591 (1453 – 1751)

APPENDIX C

Location : Raisin River
Metal : copper
Treatment: no EDTA

concentration (μgL^{-1})			cell yields (10^3)								
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	mean
Milli-Q	172	190	198	200	188	184	162	178	192	186	185
control	2742	2743	2862	2800	2828	2830	2650	2550	2675	2525	2720.5
5	2491	2548	2524	2541							2526.0
13	2352	2258	2272	2436							2305.0
20	2230	2338	2208	2268							2261.0
30	2052	2120	2188	2190							2137.5
50	1730	1756	1760	1754							1750
80	1474	1500	1446	1528							1487.0
100	1432	1398	1410	1402							1410.5
110	1261	1212	1175	1213							1215.3
120	983	944	910	944							945.25
125	705	675	647	674							675.25
200	96	94	84	86							90.0
310	30	22	28	36							29.0
500	28	22	28	20							24.5

Log concentration	I_1	I_2	I_3	I_4	mean	SD	Total
0.699	8.45	6	7.23	6.61	7.07	1.047	28.29
1.114	13.56	17.44	16.54	10.48	14.51	3.154	58.02
1.301	20.53	18.83	18.9	16.69	18.74	1.575	74.95
0.030	24.67	21.79	19.64	19.57	21.4175	2.401	85.67
1.477	36.54	35.58	35.44	35.66	35.805	0.498	143.22
1.903	46.00	45	47	44	45.50	1.528	182.00
2.000	47.53	48.77	48.36	48.65	48.33	0.559	193.31
2.041	53.84	55.62	57.02	55.61	55.52	1.303	222.09

2.079	64.09	65.55	66.76	65.55	65.49	1.092	261.95
2.097	74.34	75.48	76.5	75.5	75.46	0.883	301.82
2.301	96.83	96.9	97.26	97.2	97.0475	0.214	388.19
2.491	99.26	99.56	99.33	99.04	99.2975	0.214	397.19
2.699	99.33	99.56	99.33	99.63	99.4625	0.156	397.85

Location : Raisin River
Metal : copper
Treatment: EDTA

concentration	cell yields ($10^3 mL^{-1}$)										
(μgL^{-1})	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	mean
Milli-Q	780	880	940	968	980	1012	1016	1024	1100	1080	978
control	2428	2,292	2814	2102	2736	2608	2,320	2400	2608	2372	2468
5	2476	2,526	2462	2490							2488.5
13	2470	2,433	2607	2565							2518.75
20	2460	2,430	2470	2564							2481
30	2341	2,246	2192	2234							2253.25
50	2234	2,066	2154	2160							2153.5
80	1936	1912	2054	2090							1998
100	1572	1567	1729	1615							1620.75
110	1381	1394	1468	1372							1403.75
120	1288	1307	1328	1251							1293.5
125	1196	1220	1208	1130							1188.5
140	898	941	1587	820							1061.5
150	600	588	574	510							568
180	386	348	368	442							386
200	266	280	276	278							275
310	101	145	118	133							124.25
500	24	18	21	34							24.25

Log concentration	I1	I2	I3	I4	mean	SD
0.699	0	0	0	0	0	0
1.114	0	0	0	0	0	0
1.300	0.3	1.5	0	0	0.45	0.714
1.477	5	9	11.23	9.50	8.68	2.634
1.699	9.5	16.35	12.77	15.38	13.50	3.065
1.903	21.64	22.62	16.84	19.04	20.04	2.611
2.000	36.7	36.66	30.05	34.70	34.53	3.128
2.041	44.23	43.71	40.66	44.57	43.29	1.790
2.079	47.99	47.24	45.96	49.50	47.67	1.479
2.097	51.75	50.76	51.26	54.43	52.05	1.637
2.146	63.87	62.12	64.16	67.05	64.30	2.043
2.176	75.99	73.48	77.05	79.66	76.55	2.560
2.255	84.7	86.25	85.44	82.42	84.70	1.648
2.301	89.59	89	89.18	89.10	89.22	0.259
2.491	96.3	94.5	95.62	95.00	95.36	0.779
2.699	99.43	99.43	99.55	99.00	99.35	0.242

Location : Raisin River
Metal : Nickel
Treatment : no EDTA

concentration (µgL-1)	cell yields (10^3)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	142	208	166	178	198	198	154	162	172	178	175.6
control	2700	2752	2760	2790	2684	2600	2526	2628	2520	2620	2658
10	2360	2480	2390	2288							2379.5
25	2200	2250	2256	2280							2246.5
40	2144	2172	2133	2179							2157
63	2024	2020	2012	2006							2015.5
100	2012	1980	1880	1900							1943
150	1656	1684	1702	1698							1685
160	1627	1519	1552	1368							1516.5

200	1274	1268	1209	1227
250	1027	1005	1224	1222
300	1022	996	1165	1038
350	1025	812	826	810
400	756	814	652	592
450	447	354	394	380
630	92	80	106	100
1.000	20	24	46	54

Log conc	I1	I2	I3	I4	Mean	SD
1.00	11.25	6.7	10.12	13.97	10.51	3.010
1.39	17.3	15.41	15.18	14.27	15.54	1.272
1.60	19.41	18.35	19.83	18.09	18.92	0.833
1.69	23.94	24.09	24.4	24.62	24.26	0.306
1.78	24.4	25.6	29.38	28.63	27.00	2.383
2.00	37.84	36.78	36.1	36.25	36.74	0.788
2.20	38.94	43.01	41.77	48.72	43.11	4.110
2.30	52.27	52.49	54.72	54.04	53.38	1.191
2.40	61.59	62.42	54.15	54.23	58.10	4.525
2.50	61.78	62.76	56.38	61.18	60.53	2.839
2.55	61.67	69.71	69.18	69.79	67.59	3.954
2.60	71.83	69.64	75.76	78.02	73.81	3.779
2.65	83.5	87	85.5	86	85.5	1.472
2.80	96.9	97.36	96.37	96.6	96.81	0.428
3.00	99.62	99.47	98.64	98.34	99.02	0.541

Location : Raisin River
Metal : Nickel
Treatment : EDTA

concentration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)										mean
	R!	R2	R3	R4	R5	R6	R7	R8	R9	R10	
Milli-Q	1200	1000	1024	988	822	810	760	920	998	888	941
Control	2596	2780	2514	2092	2764	2250	2600	2214	2398	2192	2440
10	2400	2508	2412	2440							2440
30	2412	2520	2312	2460							2426
40	2380	2440	2278	2450							2389
60	2380	2420	2354	2374							2385
100	2041	1907	1930	1846							1931
150	1537	1870	1732	1754							1723
160	1900	1808	1822	1786							1829
200	1400	1570	1488	1490							1487
250	926	945	1032	1064							992
300	806	784	790	890							817.5
350	774	784	816	818							798
400	505	566	572	566							552
450	341	388	419	504							413
630	40	54	52	60							51.5
1000	24	14	20	28							21.5

Log concentration	I1	I2	I3	I4	mean	SD
1.000	1.64	0	1.15	0	0.70	0.830
1.3900	1.15	0	5.2	0	1.59	2.469
1.60	2.46	0	6.6	0	2.27	3.114
1.69	2.76	3.79	7.9	3.5	4.49	2.316
1.78	16.42	21.93	20.99	24.44	20.95	3.350
2.00	37.16	23.46	29.14	28.23	29.50	5.683
2.20	22.22	26	25.43	26.91	25.14	2.040
2.3	42.8	35.8	39.18	39.09	39.22	2.860

2.40	62.3	61.52	57.94	56.63	59.60	2.742
2.5	67.240	68.1500	67.90	63.79	66.77	2.023
2.55	68.56	68.15	66.83	66.75	67.57	0.920
2.6	79.63	77.12	76.87	77.12	77.69	1.302
2.65	86.38	84.44	83.17	79.67	83.42	2.824
2.80	98.77	98.19	98.27	97.94	90.85	0.348
3.00	99.42	99.84	99.6	99.26	99.53	0.249

Location : Ottawa River
Metal : copper
Treatment : no EDTA

concentration (μgL^{-1})	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	194	192	186	184	178	174	168	162	200	154	179.2
control	2600	2760	2558	2556	2564	2566	2606	2608	2764	2700	2628.2
5	2772	2670	2680	2696							2704.5
12.5	2372	2368	2358	2388							2371.5
20	2430	2440	2426	2446							2435.5
30	2324	2318	2330	2338							2327.5
40	1832	1834	1838	1848							1838
50	1338	1350	1346	1348							1345.5
60	1038	1061	1058	996							1038
70	653	670	668	542							633
80	246	260	260	256							255.5
100	180	190	190	256							204
130	90	94	96	82							90.5
200	34	42	36	36							37
310	15	15	20	19							27.13
500	9	8	8	9							8.5

Log concentration	I1	I2	I3	I4	mean	SD	Total
0.699	0	0	0	0	0	0.000	0.00
1.0969	9.78	9.93	10.31	9.17	9.798	0.474	39.19
1.30	7.56	7.18	7.72	6.95	7.353	0.351	29.41
1.48	11.61	11.84	11.38	10.7	11.383	0.492	45.53
1.6	30.44	30.33	30.18	29.8	30.190	0.279	120.75
1.70	49.27	48.82	48.97	48.89	48.988	0.198	195.95
1.78	63.18	62.70	62.8	65.01	63.423	1.078	253.69
1.845	77.17	76.57	76.62	81.13	77.873	2.189	311.49
1.90	90.99	90.45	90.45	97.25	92.285	3.320	369.14
2	93.97	93.62	93.59	97.25	94.608	1.770	378.43
2.11	96.94	96.79	96.72	97.25	96.925	0.235	387.70
2.30	99.08	98.78	99	99	98.965	0.129	396.89
2.49	99.81	99.81	99.62	99.66	99.725	0.099	398.90
2.70	100	100	100	100	100.000	0.000	400.00

Location: Ottawa River
Metal : copper
Treatment : EDTA

concentration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	1020	1000	1024	980	988	886	780	760	748	898	908.4
Control	2818	2800	2741	2693	2666	2766	2700	2832	2798	2754	2,756.80
5	2800	2692	2692	2728							2,728.00
10	2442	2442	2602	2604							2,522.50
20	2576	2578	2579	2582							2,578.75
30	2572	2572	2504	2506							2,538.50
50	1850	2162	1767	2162							1,985.25
60	1494	1494	1520	1520							1,507.00
70	1770	1743	1696	1734							1735.75

80	1382	1384	1258	1260							1321
100	1095	1139	1122	1046							1100.5
130	724	779	614	669							696.5
200	80	82	66	68							74
310	26	26	22	24							24.5
500	20	20	12	12							16

Log concentration	I1	I2	I3	I4	mean	SD	Total
0.699	-	2.39	2.39	1.08	1.47	1.16	5.85
1.097	11.49	11.49	5.67	5.60	8.56	3.38	34.25
1.300	6.66	6.54	6.50	6.40	6.53	0.11	26.10
1.477	11.06	11.06	4.43	4.36	7.73	3.85	30.91
1.699	21.66	21.66	22.67	22.68	22.17	0.59	31.92
1.778	33.00	35.80	36.00	35.80	35.15	1.44	88.66
1.845	46.00	46.00	45.00	45.00	45.50	0.58	148.90
1.903	50.07	49.99	54.58	54.51	52.29	2.61	209.15
2.000	60.50	58.90	59.50	62.30	60.30	1.49	296.03
2.114	74.00	72.00	78.00	76.00	75.00	2.58	382.88
2.301	97.45	97.38	97.96	97.89	97.67	0.30	390.68
2.491	99.42	99.42	99.56	99.49	99.47	0.07	397.89
2.699	99.64	99.64	99.93	99.93	99.79	99.79	399.14

Location : Ottawa River
Metal : nickel
Treatment : no EDTA

concentration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)									
	R1	R2	R3	R4	R5	R6	R7	R8	R9	MEAN
Milli-Q	200	202	186	156	154	168	178	174	190	178.89
control	2574	2440	2520	2540	2666	2800	2550	2538	2650	2586.44
0.010	2182	2180	2200	2190						2188.00
0.0250	1924	1912	1932	1928						1924.00
0.04	1882	1870	1898	1900						1887.50
0.06	1594	1586	1620	1600						1600.00
0.10	1588	1612	1588	1612						1600.00
0.16	998	1004	1006	1016						1006.00
0.25	98	98	98	106						100.00
0.40	52	60	54	58						56.00
0.63	30	26	28	28						28.00
1.00	14	12	16	14						14.00

Log concentration	I1	I2	I3	I4	mean	SD	Total
1	15.6900	15.77	14.99	15.39	15.460	0.353	61.84
1.398	25.71	26.18	25.4	25.55	25.710	0.338	102.84
1.602	27.34	27.81	26.72	26.64	27.128	0.552	108.51
1.778	38.52	38.83	37.51	38.29	38.288	0.564	153.15
2.000	38.75	37.82	38.75	37.82	38.285	0.537	153.14
2.204	61.65	61.42	61.34	60.95	61.340	0.291	245.36
2.398	96.58	96.58	96.58	96.27	96.503	0.155	386.01
2.602	98.37	98.06	98.29	98.14	98.215	0.141	392.86
2.799	99.22	99.38	99.3	99.3	99.300	0.065	397.2
3	99.84	99.92	99.77	99.84	99.843	0.061	399.37

Location : Ottawa River
Metal : nickel
Treatment : EDTA

concentration (μgL^{-1})	R1	R2	R3	R4	R5	R6	R7	R8	R9	
Milli-Q	886	1000	840	980	840	900	820	810	1020	899.56
control	2810	2728	2814	2800	2790	2815	2700	2776	2714	2771.89
10	2684	2686	2612	2666						2662.00
25	2428	2430	2430	2426						2428.50
40	2170	2172	2120	2118						2145.00
60	2354	2356	1996	1998						2176.00
100	1350	1352	1314	1314						1332.50
160	271	271	222	222						246.50
250	76	76	74	82						77.00
400	45	45	46	44						45.00
630	20	20	18	18						19.00
1000	14	14	19	19						16.50

Log concentration	I1	I2	I3	I4	mean	SD	Total
1	3.18	3.11	5.8	3.83	3.980	1.256	15.92
1.398	12.45	12.38	12.38	12.52	12.433	0.067	49.73
1.602	21.79	21.72	23.6	23.68	22.698	1.089	90.79
1.778	15.13	15.06	28.09	28.02	21.575	7.483	86.3
2.000	51.48	51.41	52.79	52.79	52.118	0.777	208.47
2.204	90.55	90.55	92.32	92.32	91.435	1.022	365.74
2.398	97.61	97.61	97.68	97.39	97.573	0.126	390.29
2.602	98.73	98.73	98.7	97.54	98.425	0.590	393.7
2.799	99.64	99.64	99.71	99.71	99.675	0.040	398.7
3	99.86	99.86	99.67	99.67	99.765	0.110	399.06

Location: Big Dam West
Metal : copper
Treatment: no EDTA

concentration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	166	158	146	140	188	200	202	198	198	180	177.6
control	400	430	406	450	474	444	446	350	398	374	417.2
5	306	308	264	298							294
12.5	210	212	212	210							211
20	116	118	80	98							103
30	62	62	72	76							68
50	38	36	40	38							68
80	14	16	14	16							15
130	20	20	20	22							20.5
200	10	12	10	12							11
310	12	14	10	12							12
500	8	12	8	4							8

Log concentration	I1	I2	I3	I4	mean I	SD	tot
0.699	27.38	26.89	37.68	29.34	30.32	5.018	121.29
1.097	50.93	50.44	50.44	50.93	50.69	0.245	202.74
1.301	73.99	73.5	82.83	78.41	77.18	3.780	308.73
1.477	87.24	87.24	84.79	83.81	85.77	1.510	343.08
1.699	93.13	93.62	92.64	93.13	93.13	0.346	372.52
1.903	99	98.53	99	98.53	98.77	0.235	395.06
2.114	97.55	97.55	97.55	97.06	97.43	0.212	389.71
2.301	100	99.51	100	99.51	99.76	0.245	399.02
2.491	99.51	99	100	99.51	99.51	0.354	398.02
2.699	100	99.51	100	100	99.88	0.212	399.51

Location: Big Dam West
Metal : copper
Treatment: EDTA

concentration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	884	772	724	890	968	972	944	880	840	780	865.4
control	1050	1016	967	1064	966	1026	960	796	894	1028	976.70
5	930	946	961	944							756.20
12.5	812	788	812	836							649.60
20	786	786	786	836							638.80
30	718	658	656	720							508.01
50	495	496	496	497							371.76
80	148	148	156	158							113.02
130	110	111	111	124							83.03
200	76	79	79	80							62.84
310	30	32	21	27							20.83
500	23	23	23	19							21.00

Log concentration	I1	I2	I3	I4	mean I	SD	tot
0.699	4.8	3.18	1.6	3.38	3.24	1.310	12.96
1.0969	17.03	19.5	17.03	14.55	17.03	2.021	68.11
1.30	19.73	19.73	19.73	14.55	18.44	3.852	73.74
1.48	26.76	32.97	33.17	35.86	32.19	3.852	128.76
1.70	49.83	49.73	49.73	49.62	49.73	0.086	198.91
1.90	85.72	85.72	84.9	84.69	85.26	0.541	341.03
2.11	89.66	89.55	89.55	88.2	89.24	0.695	356.96
2.30	93.17	92.86	92.86	92.76	92.91	0.178	371.65
2.49	97.93	97.72	98.86	98.24	98.19	0.497	392.75
2.70	98.66	98.66	98.66	99.07	98.76	0.205	395.05

Location: Big Dam West
Metal: nickel
Treatment: EDTA

Concentration (μgL^{-1})	cell yield ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	1078	1010	1024	980	978	826	988	960	890	898	963.2
Control	1080	1130	928	1062	964	1038	1140	932	1032	980	1028.60
10	832	832	826	860							837.50
25	834	835	835	836							835.00
40	802	804	802	804							803.00
60	508	508	572	572							540.00
100	310	302	294	296							300.50
160	102	102	80	80							91.00
250	38	38	36	36							37.00
400	30	28	36	36							32.50
630	24	28	26	20							24.50
1000	16	16	12	14							14.50

Log Concentration	l1	l2	l3	l4	lmean	SD	tot
1.000	19.3	19.3	19.89	16.55	18.76	1.499	75.04
1.3900	19.1	19	19	18.9	19.00	0.082	76
1.60	22.24	22.05	22.24	22.05	22.15	0.110	88.58
1.78	51.11	51.11	44.83	44.83	47.97	3.626	191.88
2.00	70.55	71.33	72.12	71.92	71.48	0.705	285.92
2.20	90.97	90.97	93.12	93.12	92.05	1.241	368.18
2.40	97.25	97.25	97.45	97.45	97.35	0.115	389.4
2.60	98.04	98.23	97.45	97.45	97.79	0.403	391.17
2.80	98.63	98.23	98.43	99	98.57	0.328	394.29
3.00	99.41	99.41	99.8	99.61	99.56	0.187	398.23

Location: Big Dam West
Metal : nickel
Treatment : no EDTA

Concentration (μgL^{-1})	cell yields ($10^{3}mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Mean
Milli-Q	150	188	182	176	144	200	174	154	198	172	173.8
Control	476	424	400	452	410	442	432	430	390	408	426.40
10	360	374	390	380							376.00
25	360	362	388	370							370.00
40	290	310	320	320							310.00
60	250	230	260	266							251.50
100	168	170	190	188							179.00
160	90	88	98	106							95.50
250	18	16	20	20							18.50
400	13	13	14	14							13.50
630	14	14	16	15							14.75
1000	13	13	14	14							13.50

Log Conc.	I1	I2	I3	I4	mean	SD	tot
1.000	15.9	12.58	8.7	11.14	12.08	3.008	48.32
1.3900	15.9	15.47	9.22	13.5	13.52	3.053	54.09
1.60	32.76	27.95	25.55	25.55	27.95	3.399	111.81
1.78	42.36	47.17	39.96	38.52	42.00	3.792	168.01
2.00	62.06	61.56	56.77	57.25	59.41	2.786	237.64
2.20	80.79	81.27	78.87	76.95	79.47	1.974	317.88
2.40	98.08	98.56	97.6	97.6	97.96	0.460	391.84
2.60	99.28	99.28	99.04	99.04	99.16	0.139	396.64
2.80	99.04	99.04	98.56	98.8	98.86	0.230	395.44
3.00	99.28	99.28	99.04	99.04	99.16	0.139	396.64

Location: Big Dam East
Metal : copper
Treatment: no EDTA

concentration (μgL^{-1})	cell yield ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	175	174	173	169	150	190	198	180	180	174	176.3
control	228	160	164	156	134	168	206	184	178	172	175
5	98	112	112	106							107
12.5	87	77	94	76							83.5
20	54	64	62	48							57
30	44	50	46	46							46.5
50	3	4	6	8							5.25
80	4	5	4	5							4.5
130	4	4	4	4							4
200	4	8	2	2							4
310	4	2	2	2							2.5
500	0	0	0	0							0

Log concentration	I1	I2	I3	I4	mean	SD	tot
0.699	46.66	38.18	38.18	41.82	41.21	4.018	164.84
1.0969	53.33	59.39	49.09	64.24	56.51	6.664	226.05
1.30	73.33	67.27	68.48	76.97	71.51	4.483	286.05
1.48	79.39	75.76	78.18	78.18	77.88	1.523	311.51
1.70	100	100	100	100	100.00	0	400
1.90	100	100	100	100	100.00	0	400
2.11	100	100	100	100	100.00	0	400
2.30	100	100	100	100	100.00	0	400
2.49	100	100	100	100	100.00	0	400
2.70	100	100	100	100	100.00	0	400

Location: Big Dam West
Metal : copper
Treatment: EDTA

concentration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	1036	1065	1080	833	738	734	820	810	790	800	870.6
control	1564	1524	1586	1636	1280	1684	1640	1280	1586	1568	1534.8
5	1378	1346	1352	1336							1353.5
12.5	1254	1224	1246	1228							1238
20	1306	1310	1240	1324							1295.1
30	488	522	466	476							488
50	124	114	110	122							117.5
80	45	38	50	48							45.25
130	30	37	42	32							35.25
200	34	32	32	36							33.5
310	20	24	28	28							25
500	12	12	14	14							13.05

Log concentration	I1	I2	I3	I4	mean I	sd	total
0.699	10.24	12.34	11.94	12.99	11.89	1.174291	
1.0969	18.37	20.34	18.9	20.08	19.48	0.940722	
1.30	14.96	14.7	19.29	13.78	15.72	2.4577	
1.48	68.64	66.4	70.08	69.42	68.62	1.60203	
1.70	92.52	93.18	93.44	97.51	92.95	2.265015	
1.9	97.7	98.16	97.38	97.51	97.65	0.341309	
2.11	98.69	98.23	97.9	86.04	98.35	6.12524	
2.30	98.43	98.56	98.56	98.29	98.44	0.128841	
2.49	99.34	99.08	98.82	98.82	99	0.248931	
2.70	99.87	99.87	99.74	99.74	99.8	0.075056	

Location: Big Dam East
Metal: nickel
Treatment: no EDTA

concentration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	166	158	146	140	188	200	202	198	198	180	177.6
control	226	150	154	152	144	168	202	150	168	162	167.6
10	136	154	153	146							147.2
25	124	127	136	140							131.5
40	68	78	92	94							73
60	90	92	94	92							92
100	78	80	84	82							81
160	24	26	28	32							25
250	20	22	24	24							21
400	18	18	18	18							18
630	16	16	18	18							16
1000	10	8	7	10							8.75

Log Concentration	l1	l2	l3	l4	mean	sd	tot
1	20.05	8.6	9.3	13.7	12.91	3.9625	51.65
1.398	27.66	25.76	20.05	17.5	22.74	3.9675	90.97
1.602	63.2	56.85	47.97	46.7	53.68	6.345	214.72
1.778	49.24	47.97	46.7	47.97	47.97	0.635	191.88
2.000	56.85	55.58	53.05	54.31	54.95	1.2675	219.79
2.204	91.17	89.85	88.58	86	88.9	1.61	355.6
2.398	93.65	92.39	91.12	91.12	92.07	0.95	368.28
2.602	94.92	94.92	94.92	94.92	94.92	0	379.68
2.799	96.16	96.16	94.92	94.92	95.54	0.62	382.16
3	100	100	100	100	62.63	0	400

Location: Big Dam East
Metal: nickel
Treatment: EDTA

concentration (μgL^{-1})	cell yields (10^3 mL^{-1})										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	1080	1028	1096	980	920	908	898	878	768	780	933.6
control	1474	1672	1512	1466	1382	1608	1316	1248	1322	1444	1444.4
10	1000	826	1060	1624							1127.5
25	1006	804	1180	1209							1049.75
40	402	600	625	800							606.75
60	400	268	258	308							308.5
100	84	84	94	78							85
160	42	52	60	54							52
250	40	38	44	34							39
400	34	18	20	20							23
630	20	16	34	22							23
1000	12	14	24	20							17.5

Log concentration	I1	I2	I3	I4	mean	sd	tot
1	30.96	43.09	26.78	0	25.21	18.17293	100.83
1.398	30.54	44.63	18.41	16.38	27.49	13.02511	109.96
1.602	72.66	58.86	77.96	44.91	63.60	14.83326	254.39
1.778	72.8	82	82.71	79.22	79.18	4.513656	316.73
2.000	94.84	94.84	94.14	95.26	94.77	0.464327	379.08
2.204	97.77	97.07	96.51	96.93	97.07	0.523832	388.28
2.398	97.91	98.05	97.63	98.33	97.98	0.291433	391.92
2.602	98.19	99.44	99.3	99.3	99.06	0.582087	396.23
2.799	99.16	99.99	98.33	99.3	99.20	0.681298	396.78
3	99.86	99.72	99.02	99.3	99.48	0.38553	397.9

Location : SLR
Metal : copper
Treatment: no EDTA

concenration (μgL^{-1})	cell yields ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	138	200	200	198	178	168	188	178	150	162	176
control	184	208	240	242	212	162	182	194	138	136	189.8
5	32	32	34	36							33.5
12.5	34	34	32	32							33
20	24	20	26	24							23.5
30	20	20	24	22							21.5
50	10	10	14	14							12
80	18	18	16	16							17
130	10	10	12	10							9
200	10	10	8	8							9
310	4	4	2	4							3.5
500	3	3	3	3							3

Log concenration	I1	I2	I3	I4	meanI	SD	tot
0.699	87.76	87.76	86.65	85.54	86.9275	1.06E+00	3.48E+02
1.0969	86.65	86.65	87.76	87.76	87.205	6.41E-01	348.82
1.30	92.26	94.43	91.1	92.26	92.5125	1.39E+00	370.05
1.48	94.43	94.43	92.26	93.33	93.6125	1.04E+00	374.45
1.70	100	100	97.78	97.78	98.89	1.281718	395.56
1.9	95.55	95.55	96.66	96.66	96.105	0.640859	384.42
2.11	100	100	98.88	100	99.72	0.56	398.88
2.30	100	100	100	100	100	0	400
2.49	100	100	100	100	100	0	400
2.70	100	100	100	100	100	0	400

Location: St. Lawrence River
Metal : copper
Treatment: EDTA

concentration	cell yields ($10^3 mL^{-1}$)										
(μgL-1)	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	1066	1080	1036	1044	1000	1000	888	980	780	800	967.4
control	3128	4192	3578	3650	3272	3608	4060	2744	3530	3529	3529.1
5	2441	2429	2646	2634							2537.5
12.5	2400	2408	2370	2294							2368
20	2320	2318	2398	2400							2359
30	2234	2360	2280	2380							2313.5
50	386	428	384	450							412
80	44	38	40	34							39
130	20	24	18	22							21
200	16	16	16	14							15.5
310	8	8	10	10							9
500	10	10	12	14							11.5

concentration	I1	I2	I3	I4	meanI	SDI	total
0.699	30.92	31.26	25.09	25.44	28.1775	3.369	112.71
1.0969	32.08	31.86	32.94	35.1	26.6154	1.479	131.98
1.30	34.36	34.42	32.14	32.08	26.86	3.077	133
1.48	36.8	33.22	32.65	32.65	27.36	3.372	135.32
1.70	89.32	88.12	89.37	87.5	71.20	7.812	354.31
1.9	99.03	99.2	99.15	99.31	79.72	8.701	396.69
2.11	99.72	99.6	99.77	99.66	80.17	8.727	398.75
2.30	99.83	88.83	99.83	99.89	78.14	9.729	388.38
2.49	100	100	100	100	80.50	8.722	400
2.70	100	100	99.97	99.89	80.51	8.700	399.86

Metal : nickel
Treatment: no EDTA

concentration (μgL^{-1}) / treatment	cell yields ($10^3 mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	180	180	186	184	190	200	172	178	168	150	178.8
control	180	184	208	208	184	238	218	172	170	172	193.4
10	124	117	122	120							120.7
25	108	108	112	106							108.14
40	100	96	96	106							99.5
60	97	94	96	92							94.79
100	91	92	94	96							93.23
160	85	83	90	92							87.7
250	78	79	84	85							81.45
400	50	50	48	46							48.5
630	30	32	28	31							30.25
1000	25	26	27	28							26.5

Log concentration	l1	l2	l3	l4	meanl	sd	tot
1	37.84	41.66	38.93	40.02	39.6125	1.629507	158.450
1.398	46.56	46.56	44.38	47.65	46.2875	1.371553	185.15
1.602	50.93	53.1	53.1	47.65	51.195	2.575222	204.78
1.778	52.56	54.2	53.1	55.29	53.7875	1.212033	215.15
2.000	55.83	55.29	54.2	53.1	54.605	1.210909	218.42
2.204	59.1	55.29	60.19	56.37	57.7375	2.28994	230.95
2.398	62.92	59.1	62.38	59.65	61.0125	1.916827	244.05
2.602	78.19	78.19	79.28	80.37	79.0075	1.043596	316.03
2.799	89.09	88	90.19	88.55	88.9575	0.934429	355.83
3	91.82	91.28	90.73	90.19	91.005	0.702306	364.02

Location: St. Lawrence River
Metal: nickel
Treatment: EDTA

concentration (μgL^{-1})	cell yields ($10^{3}mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	988	788	860	1080	1042	1122	1200	720	890	950	964
control	3200	3682	3380	3470	2520	3600	3508	3510	3620	3350	3384
10	3124	3024	2920	3012							3020
25	2541	2518	2441	2468							2492
40	2150	2110	2108	2068							2,109.00
60	1532	1434	1434	1334							1433.5
100	1128	1128	1024	1006							1071.5
160	359	334	309	306							327
250	56	56	50	50							53
400	30	36	36	34							34
630	20	20	22	24							21.5
1000	18	18	16	18							17.5

Log concentration	I1	I2	I3	I4	meanI	SDI	tot
1.000	10.36	13.29	16.23	13.59	13.368	2.401	53.47
1.3900	27.14	27.81	30.02	29.25	28.555	1.139	114.22
1.60	38.4	39.55	39.61	40.76	39.580	0.964	158.32
1.78	56.19	59	59	61.88	59.018	2.323	236.07
2.00	67.82	67.82	70.81	71.33	69.445	1.888	277.78
2.20	89.95	90.67	91.48	91.48	90.895	0.737	363.58
2.40	98.68	98.68	98.85	98.85	98.765	0.098	395.06
2.60	99.42	99.25	99.25	99.31	99.308	0.080	397.23
2.80	99.71	99.71	99.65	99.6	99.668	0.053	398.67
3.00	99.77	99.77	99.82	99.77	99.783	0.025	399.13

Location : Raisin River expose to UVB for 5 days
Metal : copper
Treatment: no EDTA

conc. (μgL^{-1})	cell yields ($x1000mL^{-1}$)										
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	177	165	135	210	155	196	186	190	198	188	180
control	3552	3690	3934	3580	3902	4446	4188	3748	2920	3772	3773
5	3735	3697	3716	3705							3713
10	3619	3548	3567	3638							3593
20	3342	3430	2446	3571							3197
25	2852	2956	2234	2639							2536
30	2362	2482	2440	2861							2536
35	1993	2086	2068	2386							2133
40	1625	1690	1696	1921							1733
45	1256	1294	1324	1456							1333
50	887	897	952	1000							934
80	153	162	232	348							224
130	20	46	42	36							36
200	34	43	22	30							32
300	33	28	14	30							26
500	16	16	14	23							17

Log conc.	I1	I2	I3	I4	mean	SD
0.699	1.00	2	1.5	2.07	1.64	0.498
1	4.08	5.99	5.42	3.85	4.83	1.035
1.3010	11.46	9.11	5.48	5.63	7.92	2.895
1.398	24.48	21.71	40.91	30.13	29.31	8.493
1.477	37.50	34.30	35.43	24.5	32.93	5.774
1.544	47.30	44.83	45.31	36.86	43.57	4.602
1.602	57.10	55.36	55.2	49.22	54.22	2.981
1.653	66.90	65.89	65.09	61.58	64.87	2.312
1.699	76.70	76.43	74.97	73.94	75.51	1.293
1.903	86.45	86.19	84.54	82.61	84.95	1.773
2.114	96.20	95.96	94.1	91.29	94.39	2.268

2.301	99.72	99.05	99.15	99.29	99.30	0.297
2.477	99.34	99.12	99.68	99.74	99.47	0.293
2.699	99.84	99.82	99.9	99.92	99.87	0.048

Raisin River expose to UVB for 5 days
Metal: nickel
Treatment: no EDTA

Conc (μgL^{-1})	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	173.5	210	133	145	188	198	168	156	178	186	173.56
control	3780	3678	3898	3858	3820	3898	3878	3920	3880	3851	3851.00
10	3220	3100	3122	3000							3110.50
25	2800	2790	2740	2724							2763.50
40	2280	2268	2254	2266							2267.00
60	2180	2168	2196	2182							2181.50
100	1968	1994	1940	1972							1968.50
160	1930	1950	1940	1900							1930.00
250	1750	1742	1724	1770							1746.50
400	758	742	770	762							758.00
630	118	114	120	116							117.00
1000	82	76	84	84							81.50

Log Conc.	I1	I2	I3	I4	mean	SD
1.00	16.43	19.55	18.98	22.16	19.28	2.35
1.3900	27.36	27.62	28.92	29.34	28.31	0.97
1.60	40.90	41.21	41.58	29.34	38.26	5.95
1.78	43.50	43.82	43.09	43.45	43.47	0.30
2.00	49.02	48.35	49.75	48.92	49.01	0.57
2.20	50.00	49.49	49.75	50.79	50.01	0.56
2.40	54.70	54.91	54.91	54.18	54.68	0.34
2.60	80.53	80.94	80.21	80.42	80.53	0.31
2.80	97.19	97.29	96.87	97.24	97.15	0.19
3.00	98.125	98.28	98.07	98.07	98.14	0.10

Location : Raisin River expose to UVB for 10 days
Metal : copper
Treatment: no EDTA

conc	cell yields (x1000mL^{-1})										
µgL^{-1}	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	mean
Milli-Q	190	254	188	166	174	158	166	200	222	220	193.8
control	3450.00	3678.00	3900.00	3840.00	3690.00	3700.00	3724.00	3560.00	3690.00	3598.00	3683
0.5	3386.00	3340.00	3420.00	3382.00							3382
1.3	3212.00	3198.00	3144.00	3190.00							3186
2	2828.00	2868.00	2750.00	2866.00							2828
3	1631.00	1683.00	1640.00	1678.00							1658
5	1100.00	1264.00	1130.00	1146.00							1160
8	300.00	240.00	260.00	280.00							270
12.5	140.00	128.00	138.00	150.00							139
20	78.00	68.00	58.00	58.00							66
31.5	12	12	12	10							12
50	10	10	8	6							9
130	10	8	2	4							6
200	10	6	2	2							5
300	0	0	0	0							0
500	0	0	0	0							0

Log Conc.	I1	I2	I3	I4	mean	SD
-0.301	8.807	9.34	7.16	8.19	8.37	0.94
0.114	12.82	13.2	14.67	13.42	13.53	0.80
0.301	23.28	22.189	25.4	22.24	23.28	1.50
0.477	55.867	54.45	55.62	54.59	30.44	0.72
0.699	70.32	65.86	69.51	69.07	68.69	1.96
0.903	92.1	93.74	93.19	96.19	93.80	1.73
1.096	96.46	96.78	96.52	96.19	96.49	0.24
1.301	98.15	98.42	98.69	96.69	96.49	0.89
1.498	99.95	99.95	99.95	100	99.96	0.02
1.699	100	100	100	100	100	0
2.114	100	100	100	100	100	0

2.301	100	100	100	100	100	0
2.477	100	100	100	100	100	0
2.699	100	100	100	100	100	0

Location : Raisin River expose to UVB for 10 days
Metal : nickel
Treatment : no EDTA

conc.	cell yields (x1000mL^{-1})										
µgL^{-1}	r1	r2	r3	r4	r5	R6	R7	R8	R9	R10	
Milli-Q	184	180	196	222	104	180	132	210	212	242	186.2
control	3430	3576	3889	3952	3380	3652	3422	3990	3224	3612	3612.7
10	2742	2358	2818	2682							2650.0
25	2279	2248	2888	2366							2445.3
40	2230	1904	2572	2505							2302.8
60	1955	1752	1837	1750							1823.5
100	1544	1798	1768	1572							1670.5
160	1272	1488	1210	1442							1353.0
250	614	596	562	611							595.8
400	200	198	218	216							208.0
630	92	108	110	100							102.5
1000	54	56	51	59							55.0

Log conc	I1	I2	I3	I4	mean	SD
1.00	24.17	34.83	22.06	25.83	26.7	5.62
1.39	37.09	37.88	20.12	34.05	32.3	8.28
1.60	38.38	47.43	28.89	30.75	36.4	8.44
1.78	46.01	51.65	49.29	51.7	49.7	2.68
2.00	57.42	50.37	51.2	56.64	53.9	3.64
2.20	64.97	58.98	66.69	60.25	62.7	3.69
2.40	83.23	83.73	84.68	83.32	83.7	0.66
2.60	94.73	94.78	94.23	94.28	94.5	0.29
2.80	97.72	97.3	97.22	97.5	97.4	0.22
3.00	98.78	98.72	98.86	98.64	98.8	0.09

Organism: **Daphnia magna**
Location : Raisin River
Metal : copper

Concentration (mgL-1)	RI	r2	R3	pH before	pH after	DO before	DO after
1.5	10	10	10	8.3	8.58	5	5
0.9	10	10	10	8.3	8.59	5	5
0.6	9	6	8	8.3	8.5	5	5
0.5	4	4	3	8.3	8.56	5	5
0.15	0	0	0	8.2	8.59	5	5
0.015l	0	0	0	8.2	8.59	5	5
0.005	0	0	0	8.2	8.59	5	5
Control	0	0	0	8.2	8.59	5	5

Location: Raisin River
Metal : nickel

Concentration (mgL^{-1})	R1	R2	R3	pH before	pH after	DO before	DO after
4	7	8	8	8.29	8.32	5	5
3.5	5	6	5	8.26	8.66	5	5
3	5	4	5	8.26	8.65	5	5
2.5	1	3	3	8.26	8.64	5	5
1.5	1	2	2	8.28	8.64	5	5
1	1	0	0	8.28	8.76	5	5.2
0.46	0	0	0	8.26	8.81	5	5.2
0.23	0	0	0	8.26	8.77	5	5.2
0.11	0	0	0	8.26	8.79	5	5.2
0.046	0	0	0	8.28	8.88	5	5.2
0.023	0	0	0	8.28	8.81	5	5.2
0.01	0	0	0	8.28	8.81	5	5.2
control	0	0	0	8.3	8.84	5	5.2

Location : Ottawa River
Metal : copper

Concentration (mgL^{-1})	RI	r2	R3	pH before	pH after	DO before	DO after
1	10	10	10	7.49	7.6	5	5
0.46	10	10	10	7.49	7.7	5	5.2
0.22	10	10	10	7.4	7.8	5	5
0.11	10	10	10	7.4	7.6	5	5.2
0.05	8	5	4	7.42	7.6	5	6
0.04	5	4	4	7.43	7.7	5	5
0.023	0	0	0	7.4	7.6	5	5
0.011	0	0	0	7.4	7.6	5	5

Location : Ottawa River
Metal : nickel

Concentration (mgL^{-1})	R1	R2	R3	ph before	pH after	DO before	DO after
2	6d	8d	6d	7.44	7.82	5	5
1.5	6d	5d	5d	7.34	7.75	5	5
0.945	2d	2d	1d	7.27	7.69	5	5
0.69	1	0	0	7.28	7.48	5	5
0.33	0	0	1	7.4	7.5	5	5
0.23	0	0	1	7.4	7.5	5	5
0.15	0	0	0	7.4	7.4	5	5
0.095	0	0	0	7.4	7.4	5	5
0.069	0	0	0	7.4	7.4	5	5
control	0	0	0	7.4	7.4	5	5

Location : St. Lawrence River
Metal : copper

Concentration (mgL^{-1})	RI	R2	R3	pH before	pH after	DO before	DO after
0.5	10	10	10	8.2	8.35	6	6
0.23	10	10	10	8.2	8.35	6	6
0.11	10	10	9	8.2	8.33	6	6
0.05	3	3	4	8.2	8.32	6	6
0.023	0	0	0	8.2	8.32	6	6
0.011	0	0	0	8.2	8.33	6	6
0.005	0	0	0	8.2	8.35	6	6
control	0	0	0	8.2	8.33	6	6

Location : St. Lawrence River
Metal : nickel

Concentration (mgL^{-1})	R1	R2	R3	pH before	pH after	DO before	DO after
2	7	8	8	8.2	8.48	6	6
1.5	5	5	4	8.2	8.48	6	6
0.92	0	1	0	8.2	8.49	6	6
0.33	0	0	0	8.2	8.48	6	6
0.15	0	0	0	8.2	8.48	6	6
0.095	0	0	0	8.2	8.48	6	6
0.069	0	0	0	8.2	8.48	6	6
control	0	0	0	8.1	8.49	6	6

Location : Big Dam West lake
Metal : copper

Concentration (mgL^{-1})	RI	R2	R3	pH before	pH after	DO before	DO after
0.5	10	10	10	7.2	7.31	5	4.8
0.23	10	10	10	7.2	7.21	5	4.8
0.11	10	10	10	7.2	7.21	5	5
0.05	8	7	7	7.2	7.41	5	5.4
0.023	4	2	2	7.2	7.5	5	4.4
0.011	1	0	0	7.2	7.4	5	5.4
0.005	0	0	0	7.2	7.4	5	5.4
control	0	0	0	7.4	7.4	5	5.4

Location : Big Dam West lake
Metal : nickel

Concentration (mgL^{-1})	R1	R2	R3	pH before	pH after	DO before	DO after
1.5	10	10	10	7.2	7.4	5	5
1	10	10	10	7.2	7.4	5	5
0.69	10	10	10	7.2	7.4	5	5
0.33	7	7	8	7.2	7.4	5	5
0.25	6	6	8	7.2	7.4	5	5
0.2	3	3	4	7.2	7.4	5	5
0.15	0	0	0	7.2	7.3	5	5
0.09	0	0	0	7.2	7.3	5	5
0.069	0	0	0	7.2	7.3	5	5
control	0	0	0	7.2	7.3	5	5

Location : Big Dam East Lake
Metal : copper

Concentration (mgL^{-1})	RI	r2	R3	pH before	pH after	DO before	DO after
0.5	10	10	10	7.49	7.4	5	5
0.23	10	10	10	7.25	7.4	5	5
0.11	10	10	10	7.27	7.4	5	5
0.05	8	8	8	7.2	7.4	5	5
0.023	4	7	6	7.2	7.4	5	5
0.011	2	2	2	7.2	7.4	5	5
0.005	0	0	0	7.2	7.4	5	5
control	0	0	0	7.24	7.55	5	5.2
medium	0	0	0	7.8	8.66	5	5

Location : Big Dam East Lake
Metal : copper

Concentration ($^{mgL-1}$)	R1	R2	R3	pH before	pH after	DO before	DO after
1.5	10	10	10	7.69	7.4	5	5
0.945	10	10	10	7.5	7.4	5	5
0.69	10	10	10	7.64	7.4	5	5
0.33	6	6	8	7.2	7.4	5	5
0.2	4	4	6	7.2	7.4	5	5
0.15	0	0	0	7.2	7.4	5	5
0.095	0	0	0	7.2	7.4	5	5
0.069	0	0	0	7.2	7.4	5	5
control	0	0	0	8.07	7.4	5	5

Appendix D
Free Ion Activity Model

Together with the interaction of metal and aquatic organisms, Morel (1983) formulated the free ion activity model (FIAM), a model based on the several equilibrium steps.

a. solution equilibrium

$$M^{z+} + L \overset{K_1}{\leftrightarrow} ML, \text{ where } K_1 = [ML] / [M^{z+}][L] \quad (1)$$

b. surface reaction of M^{z+}:

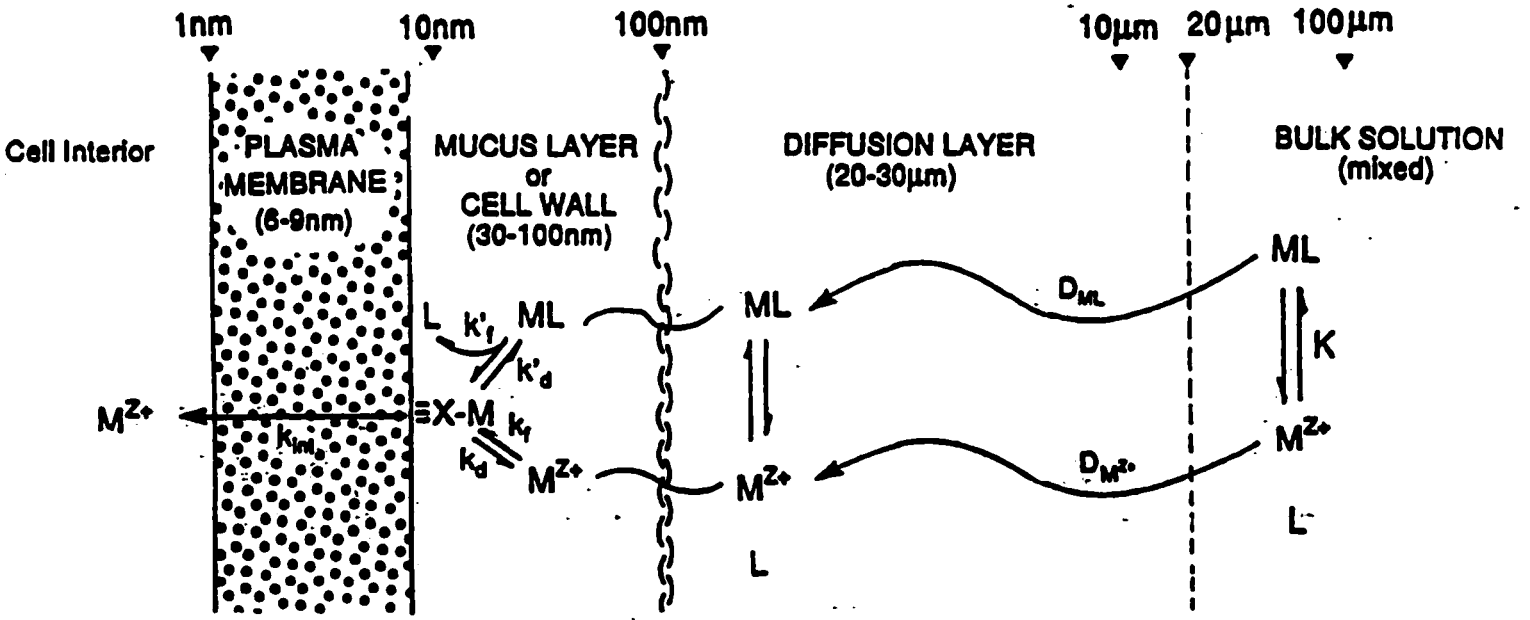
$$M^{z+} + \bar{X}\text{-cell} \overset{K_2}{\leftrightarrow} M\text{-X-cell}, \text{ where } \{M\text{-X-cell}\} = K_2\{\bar{X}\text{-cell}\}[M^{z+}] \quad (2)$$

c. surface reaction of ML

$$ML + \bar{X}\text{-cell} \overset{K_3}{\leftrightarrow} M\text{-X-cell} + L, \text{ where } \{M\text{-X-cell}\} = K_3\{\bar{X}\text{-cell}\}[ML]/[L] \quad (3)$$

Conceptual model of metal-organisms interaction (Campbell 1995)

M^{z+} = free metal ion; ML = metal complex in solution; M-X-membrane: surface metal complex; k_f = rate constants for formation of the surface complex; K_d = rate constant for internalization



APPENDIX E
STATISTICAL ANALYSES

Chapter 2

One-way ANOVA

- Ho : There is no significant IC50 of copper for *S. capricornutum* in all locations

Dep Var	N	Factors	df	F-ratio	P
L10IC50	12	LOCATION	4	120.185	0 *

Post Hoc test (pairwise comparison prob.)

Comparison	Bonferoni	Tukey	Scheffe	LSD
BDE-BDW	1	0.998	0.999	0.771
BDE-SLR	0*	0.000*	0.000*	0.000*
BDE-OR	0.001*	0.001*	0.000*	0.000*
BDE-RR	0.000*	0.000*	0.000*	0.000*
BDW-SLR	0.000*	0.000*	0.000*	0.000*
BDW-OR	0.000*	0.000*	0.000*	0.000*
BDW-RR	0.000*	0.000*	0.000*	0.000*
SLR-OR	0.000*	0.000*	0.000*	0.000*
SLR-RR	0.000*	0.000*	0.000*	0.000*
OR-RR	0.081	0.045*	0.078	0.008*

- Ho: There is no significant IC50 of Ni for *S. capricornutum* in all locations.

Dep Var	N	Factors	df	F-ratio	P
L10IC50	14	LOCATION	4	8.699	0.004 *

Post Hoc test (pairwise comparison prob.)

Comparison	Bonferoni	Tukey	Scheffe	LSD
BDE-BDW	1.000	0.921	0.951	0.439
BDE-SLR	1.000	1.000	1.000	0.921
BDE-OR	1.001	0.778	0.851	0.280
BDE-RR	0.006*	0.004*	0.009*	0.001*
BDW-SLR	1.000	0.968	0.981	0.549
BDW-OR	1.000	0.997	0.998	0.742
BDW-RR	0.019*	0.012*	0.025*	0.002
SLR-OR	0.000*	0.880	0.924	0.378
SLR-RR	0.015*	0.010*	0.000*	0.001*
OR-RR	0.031*	0.020*	0.039*	0.003*

- Ho: There is no different of LC50 of Cu for *D. magna* in all locations

Dep Var	N	Factors	df	F-ratio	P
LC50	13	LOCATION	4	1294.708	0.000

Post Hoc test (pairwise comparison prob.)

Comparison	Bonferoni	Tukey	Scheffe	LSD
BDE-BDW	1.000	0.983	0.990	0.616
BDE-SLR	0.007*	0.005*	0.010*	0.001*
BDE-OR	0.230	0.121	0.193	0.023*
BDE-RR	0.000*	0.000*	0.000*	0.000*
BDW-SLR	0.014*	0.009*	0.017*	0.001*
BDW-OR	0.518	0.243	0.346	0.052
BDW-RR	0.000*	0.000*	0.000*	0.000*
SLR-OR	0.360	0.179	0.268	0.036*
SLR-RR	0.000*	0.000*	0.000*	0.000*
OR-RR	0.000*	0.000*	0.000*	0.000*

- Ho: There is no different of LC50 of Ni for *D. magna* in all locations

Dep Var	N	Factors	df	F-ratio	P
LC50	13	LOCATION	4	146.022	0.000

Post Hoc test (pairwise comparison prob.)

Comparison	Bonferoni	Tukey	Scheffe	LSD
BDE-BDW	1.000	0.998	0.99	0.791
BDE-SLR	0.000*	0.000*	0.000*	0.000*
BDE-OR	0.000	0.000	0.000	0.000*
BDE-RR	0.000*	0.000*	0.000*	0.000*
BDW-SLR	0.000*	0.000*	0.000*	0.001*
BDW-OR	0.000	0.000	0.000	0.000
BDW-RR	0.000*	0.000*	0.000*	0.000*
SLR-OR	1.000	1.000	1.000	0.845*
SLR-RR	0.000*	0.000*	0.000*	0.000*
OR-RR	0.000*	0.000*	0.000*	0.000*

Sensitivity organisms

- H0 : There is no significant sensitivity of organisms.

Kruskal Wallis- one way Non parametric

Dep Var	N	Factors	df	F-ratio	P	M-whithey	Dm	Sc	Location
LC50	60	pool	1		0.000*	696.000			general
LC50	30	Ni	1		0.000*	222.000	342.000	123.000	general
LC50	30	Cu	1		0.024*	167.000	287.000	178.000	general
LC50	12	pool	1	8.308	0.004*	36.000	57.000	21.000	RR
LC50	12	pool	1		0.337	24.000	45.000	33.000	OR
LC50	12	pool	1	2.077	0.150	27.000	48.000	30.000	BDWL
LC50	12	pool	1	5.769	0.016*	33.000	54.000	24.000	SLR
LC50	12	pool	1	2.077	0.150	27.000	48.000	30.000	BDEL
LC50	6	Cu	1	3.857	0.050*	0.0	6.000	15.000	RR
LC50	6	Cu	1	3.857	0.050*	0.0	6.000	15.000	OR
LC50	6	Cu	1	3.857	0.050*	0.0	6.000	15.000	BDWL
LC50	6	Cu	1	3.857	0.050*	0.0	6.000	15.000	SLR
LC50	6	Cu	1	3.857	0.050*	0.0	6.000	15.000	BDEL
LC50	6	Ni	1	3.857	0.050*	0.0	6.000	15.000	RR
LC50	6	Ni	1	3.857	0.050*	0.000	6.000	15.000	OR
LC50	6	Ni	1	3.857	0.050*	0.0	6.000	15.000	BDWL
LC50	6	Ni	1	3.857	0.050*	0.0	6.000	15.000	SLR
LC50	6	Ni	1	3.857	0.050*	0.0	6.000	15.000	BDEL

The toxicity of Cu and Ni

Ho : There is no significant toxicity between copper and nickel

Kruskal Wallis One-way -nonparametric

Dep Var	N	Factors	df	$\chi 2$	P	M-whithey	Cu	Ni	Location
LC50	60	pool	1	20.735	0.000*	142.000	607.000	1223.000	general
LC50	30	Dm	1	15.364	0.000	18.000	138.000	327.000	general
LC50	30	Sc	1	8.551	0.003	42.000	162.000	303.000	general
LC50	12	pool	1	2.077	0.150	9.000	30.000	48.000	RR
LC50	12	pool	1	5.026	0.025*	4.000	25.000	53.000	OR
LC50	12	pool	1	8.308	0.005*	27.000	21.000	57.000	BDWL
LC50	12	pool	1	3.692	0.004*	33.000	27.000	51.000	SLR
LC50	12	pool	1	8.308	0.004*	0.0	21.000	57.000	BDEL

LC50	6	Sc	1	3.857	0.050*	0.0	6.000	15.000	RR
LC50	6	Sc	1	1.190	0.275	2.000	6.000	15.000	OR
LC50	6	Sc	1	3.857	0.050*	0.0	6.000	15.000	BDWL
LC50	6	Sc	1	3.857	0.050*	0.0	6.000	15.000	SLR
LC50	6	Sc	1	3.857	0.050*	0.0	6.000	15.000	BDEL
LC50	6	Dm	1	3.857	0.050*	0.0	6.000	15.000	RR
LC50	6	Dm	1	3.857	0.050*	0.000	6.000	15.000	OR
LC50	6	Dm	1	3.857	0.050*	0.0	6.000	15.000	BDWL
LC50	6	Dm	1	3.857	0.050*	0.0	6.000	15.000	SLR
LC50	6	Dm	1	3.857	0.050*	0.0	6.000	15.000	BDEL

- * significant

Chapter 3

Control for copper or nickel test:
Kruskal Wallis One way analysis for N=20

- Ho : There is no significance the algal growth control for copper and nickel toxicity.

treatments	$\chi 2$	df	probability	Mann-Whitney -U
0 days	1.651	1	0.199	67.000 (accept the null)
5 days	0.366	1	0.545	42.000 (accept the null)
10 days	0.823	1	0.364	62.000 (accept the null)

- Ho: There is no significance the effect of UV-B exposure on the control of algal growth for copper and nickel toxicity.

One way ANOVAa

Dep Var.	N	F-ratio	P
Cell yield	58	290.379	0.000 (reject the null)

Post Hoc Test of cell yield (pairwise mean differences)

	0-5	0-10	5-10
Bonferoni	0.000*	0.000*	0.004*
Scheffe	0.000*	0.000*	0.005*
Tukey	0.000*	0.000*	0.003*
LSD	0.000*	0.000*	0.001*

- There is significance cell yields on the control of copper and nickel test at 0, 5 and 10 days UV-B exposure.

Main statistic One way ANOVA

- Ho : there is no significance the length of UV-B exposure to the algal cell growth

Metal	Dep. Variable	N	F-ratio	P
Copper	IC50	9	23.760	0.001* (reject the null)
Nickel	IC50	9	20.085	0.002* (reject the null)

Post Hoc test of IC50 (pairwise mean differences:) for copper

	0-5	0-10	5-10
Bonferoni	0.015*	0.001*	0.138
Scheffe	0.015*	0.001*	0.116
Tukey	0.012*	0.001*	0.101
LSD	0.005*	0.000*	0.046*

- There is a significance effect of the length of exposure between 0 and 5, 0 and 10.
- There is no significance effect of the length of exposure between 5 and 10 days in copper toxicity, except for LSD.

Post Hoc test of IC50 (pairwise mean differences:) for nickel

	0-5	0-10	5-10
Bonferoni	0.010*	0.003*	0.665
Scheffe	0.010*	0.003*	0.445
Tukey	0.008*	0.002*	0.415
LSD	0.003*	0.001*	0.222

- There is significance effect of the length of exposure between 0 and 5, 0 and 10 in nickel toxicity.
- There is no significance effect of the length of exposure between 5 and 10 days in nickel toxicity

Chapter 4

- H0 : There was no significance on the cell yield of control for copper and nickel toxicity in each location.

Location	Treatment	$\chi 2$	df	probability	Mann-Whitney U stat
RR	EDTA	0.28	1	0.597	57.000 (accept the null)
RR	no-EDTA	1.651	1	0.199	67.000 (accept the null)
OR	EDTA	0.023	1	0.880	48.000 (accept the null)
OR	no-EDTA	1.286	1	0.257	65.000 (accept the null)
BDWL	EDTA	2.766	1	0.096	28.000 (accept the null)
BDWL	no-EDTA	1.286	1	0.257	65.000 (accept the null)
BDEL	EDTA	2.647	1	0.104	71.500 (accept the null)
BDEL	no-EDTA	0.206	1	0.65	44.000 (accept the null)
SLR	EDTA	0.823	1	0.364	62.000 (accept the null)
SLR	no-EDTA	0	1	1	50.000 (accept the null)

Copper Toxicity Test
By Two-way parametric-factorial anova, model I

H01 : There were no significance on copper LC50 among locations
HO2 : copper IC50 with addition EDTA is lower than that without EDTA
Ho3 : There were not interaction of location and EDTA

Dep Var:	N	factors	df	F-ratio	P
L10LC50	27	LOCATION	4	93.186	0.000* (reject the null)
		EDTA	1	156.425	0.000 *(0/2, reject the null)
		EDTA*LOCAT	4	21.32	0.000* (reject the null)

Power (1-β)

The role of EDTA on the IC50 for each location
Kruskal-Wallis One-Way Analysis of Variance for 6 cases

Location	$\chi 2$	df	prob	Mann-Whitney-U stat
RR	0.429	1	0.513	3.000
OR	3.857	1	0.05*	0
BDWL	3.857	1	0.05*	0
BDEL	3.857	1	0.05*	0
SLR	3.857	1	0.05*	0

Nickel Toxicity Test
By two-way non parametric

H01 : There were no significance on copper LC50 among locations
HO2 : There were no significance on copper LC50 between addition and omission of EDTA
Ho3 : There were not interaction of location and EDTA

Dep Var:	N	Factors	SS	df	H	P
IC50	30	Location	125106.018	4	26.967	< 0.001**
		EDTA	0.550	1	0.0001	>0.005
		EDTA*LOC	2298.426	4	0.495	>0.005

Concl:
Reject the H01, there was significance on nickel LC50 among locations.
Accept the H02, there were not significance on nickel toxicity between addition and omission of EDTA.
Accept the H03, there were not interaction locations and EDTA treatment.

Power (1-β) =

The role of EDTA on the IC50 for each location
Kruskal-Wallis One-Way Analysis of Variance for 6 cases

Location	$\chi 2$	df	prob	Mann-Whitney-U stat
RR	2.333	1	0.127	1.000
OR	0.048	1	0.827	4.000
BDWL	0.048	1	0.827	4.000
BDEL	3.857	1	0.05*	0
SLR	0.196	1	0.658	5.500

APPENDIX F
OUTPUT WHAM

SOURCE FILE	RR 0 day (copper)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00

INPUT DATA

TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	2.440E-02
PCO2	3.500E-04

MASTER SPECIES	TOTAL CONC
3 Na	3.900E-04
4 Mg	2.800E-04
5 Al	3.700E-07
6 K	2.900E-06
7 Ca	2.170E-03
9 Mn	1.500E-06
10 Fe	1.700E-05
12 Co	3.800E-11
13 Ni	3.300E-11
14 Cu	4.950E-07
15 Zn	1.500E-08
17 Cd	8.900E-12
19 Ba	1.400E-11
52 Cl	1.910E-04
53 NO3	1.800E-04
54 SO4	1.000E-04
57 PO4	3.700E-07

****

RESULTS

NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.605E-03
CHARGE RATIO	4.426E+00
CHARGE DIFFERENCE	+3.981E-03
ZED-FA	-2.309E-03
RATIO-FA	1.879E+00

WATER VOLUMES

FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	3.221E-03
FRACTION SOLUTION	0.997E+00
CARBONATE ALKALINITY	+6.260E-04

****

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES	S	DHA	DFA	HA	FA
3 Na	0.994	0.000	0.006	0.000	0.000
4 Mg	0.977	0.000	0.011	0.000	0.012
5 Al	0.973	0.000	0.000	0.000	0.027
6 K	0.994	0.000	0.006	0.000	0.000
7 Ca	0.977	0.000	0.011	0.000	0.012
9 Mn	0.924	0.000	0.010	0.000	0.066
10 Fe	0.377	0.000	0.003	0.000	0.619
12 Co	0.954	0.000	0.008	0.000	0.039
13 Ni	0.752	0.000	0.005	0.000	0.243
14 Cu	0.019	0.000	0.000	0.000	0.980
15 Zn	0.379	0.000	0.004	0.000	0.617
17 Cd	0.751	0.000	0.008	0.000	0.240
19 Ba	0.985	0.000	0.011	0.000	0.004
52 Cl	1.000	0.000	0.000	0.000	0.000
53 NO3	1.000	0.000	0.000	0.000	0.000
54 SO4	0.999	0.000	0.001	0.000	0.000
57 PO4	0.999	0.000	0.001	0.000	0.000

****

SOURCE FILE	RR 5 days (copper)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00
INPUT DATA	
TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	2.120E-02
PCO2	3.500E-04
RESULTS	
NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.619E-03
CHARGE RATIO	4.440E+00
CHARGE DIFFERENCE	+3.996E-03
ZED-FA	-2.298E-03
RATIO-FA	1.875E+00

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

	MASTER SPECIES	S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.980	0.000	0.010	0.000	0.010
5	Al	0.979	0.000	0.000	0.000	0.021
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.980	0.000	0.010	0.000	0.010
9	Mn	0.936	0.000	0.009	0.000	0.056
10	Fe	0.436	0.000	0.003	0.000	0.560
12	Co	0.961	0.000	0.007	0.000	0.033
13	Ni	0.792	0.000	0.005	0.000	0.203
14	Cu	0.025	0.000	0.000	0.000	0.975
15	Zn	0.438	0.000	0.004	0.000	0.558
17	Cd	0.790	0.000	0.008	0.000	0.203
19	Ba	0.987	0.000	0.010	0.000	0.003
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

****

SOURCE FILE	RR 10 days (copper)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00
INPUT DATA	
TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	1.960E-02
PCO2	3.500E-04
RESULTS	
NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.626E-03
CHARGE RATIO	4.447E+00
CHARGE DIFFERENCE	+4.003E-03
ZED-FA	-2.293E-03
RATIO-FA	1.872E+00

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

	MASTER SPECIES	S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.982	0.000	0.009	0.000	0.009

5	Al	0.982	0.000	0.000	0.000	0.018
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.982	0.000	0.009	0.000	0.009
9	Mn	0.941	0.000	0.008	0.000	0.051
10	Fe	0.468	0.000	0.003	0.000	0.528
12	Co	0.964	0.000	0.006	0.000	0.030
13	Ni	0.812	0.000	0.004	0.000	0.183
14	Cu	0.028	0.000	0.000	0.000	0.971
15	Zn	0.471	0.000	0.004	0.000	0.526
17	Cd	0.808	0.000	0.007	0.000	0.185
19	Ba	0.988	0.000	0.009	0.000	0.003
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

****

SOURCE FILE	RR 0 days (Nickel)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00
INPUT DATA	
TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	2.440E-02
PCO2	3.500E-04

MASTER SPECIES	TOTAL CONC
3 Na	3.900E-04
4 Mg	2.800E-04
5 Al	3.700E-07
6 K	2.900E-06
7 Ca	2.170E-03
9 Mn	1.500E-06
10 Fe	1.700E-05
12 Co	3.800E-11
13 Ni	6.450E-07
14 Cu	1.600E-11
15 Zn	1.500E-08
17 Cd	8.900E-12
19 Ba	1.400E-11
52 Cl	1.910E-04
53 NO3	1.800E-04
54 SO4	1.000E-04
57 PO4	3.700E-07

RESULTS	
NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.605E-03
CHARGE RATIO	4.427E+00
CHARGE DIFFERENCE	+3.981E-03
ZED-FA	-2.309E-03
RATIO-FA	1.879E+00
WATER VOLUMES	
FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	3.220E-03
FRACTION SOLUTION	0.997E+00
CARBONATE ALKALINITY	+6.260E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES	S	DHA	DFA	HA	FA
3 Na	0.994	0.000	0.006	0.000	0.000
4 Mg	0.977	0.000	0.011	0.000	0.012
5 Al	0.972	0.000	0.000	0.000	0.028
6 K	0.994	0.000	0.006	0.000	0.000
7 Ca	0.977	0.000	0.011	0.000	0.012
9 Mn	0.924	0.000	0.010	0.000	0.067

10	Fe	0.367	0.000	0.003	0.000	0.629
12	Co	0.953	0.000	0.008	0.000	0.039
13	Ni	0.745	0.000	0.005	0.000	0.250
14	Cu	0.019	0.000	0.000	0.000	0.981
15	Zn	0.369	0.000	0.003	0.000	0.627
17	Cd	0.746	0.000	0.008	0.000	0.246
19	Ba	0.985	0.000	0.011	0.000	0.004
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

****

SOURCE FILE RR 5 days (Nickel)
DATABASE water10
PH FIXED
PRECISION % 1.000E-02
STARTING PH 8.000E+00
INPUT DATA

TEMPK 2.930E+02
TOT HA 0.000E+00
TOT FA 2.120E-02
PCO2 3.500E-04

RESULTS
NO. OF ITERATIONS 50
PH 8.000E+00
IONIC STRENGTH 5.619E-03
CHARGE RATIO 4.440E+00
CHARGE DIFFERENCE +3.996E-03
ZED-FA -2.297E-03
RATIO-FA 1.875E+00
WATER VOLUMES
FRACTION HA-DDL 0.000E+00
FRACTION FA-DDL 2.789E-03
FRACTION SOLUTION 0.997E+00
CARBONATE ALKALINITY +6.264E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES		S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.980	0.000	0.010	0.000	0.010
5	Al	0.978	0.000	0.000	0.000	0.022
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.980	0.000	0.010	0.000	0.010
9	Mn	0.935	0.000	0.009	0.000	0.056
10	Fe	0.425	0.000	0.003	0.000	0.572
12	Co	0.960	0.000	0.007	0.000	0.033
13	Ni	0.786	0.000	0.005	0.000	0.210
14	Cu	0.024	0.000	0.000	0.000	0.976
15	Zn	0.427	0.000	0.003	0.000	0.570
17	Cd	0.784	0.000	0.008	0.000	0.208
19	Ba	0.987	0.000	0.010	0.000	0.003
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

****

SOURCE FILE RR 10 days (nickel)
DATABASE water10
PH FIXED
PRECISION % 1.000E-02
STARTING PH 8.000E+00

INPUT DATA
TEMPK 2.930E+02
TOT HA 0.000E+00
TOT FA 1.900E-02

PCO2	3.500E-04

RESULTS

NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.629E-03
CHARGE RATIO	4.450E+00
CHARGE DIFFERENCE	+4.006E-03
ZED-FA	-2.289E-03
RATIO-FA	1.871E+00
WATER VOLUMES	
FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	2.495E-03
FRACTION SOLUTION	0.998E+00
CARBONATE ALKALINITY	+6.266E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES		S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.982	0.000	0.009	0.000	0.009
5	Al	0.982	0.000	0.000	0.000	0.018
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.982	0.000	0.009	0.000	0.009
9	Mn	0.943	0.000	0.008	0.000	0.050
10	Fe	0.468	0.000	0.003	0.000	0.529
12	Co	0.965	0.000	0.006	0.000	0.029
13	Ni	0.813	0.000	0.004	0.000	0.183
14	Cu	0.028	0.000	0.000	0.000	0.972
15	Zn	0.470	0.000	0.003	0.000	0.526
17	Cd	0.810	0.000	0.007	0.000	0.183
19	Ba	0.988	0.000	0.009	0.000	0.003
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	1.000	0.000	0.000	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

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SOURCE FILE	Copper IC50
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00

INPUT DATA

TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	2.440E-02
PCO2	3.500E-04

MASTER SPECIES		TOTAL CONC
3	Na	3.900E-04
4	Mg	2.800E-04
5	Al	3.700E-07
6	K	2.900E-06
7	Ca	2.170E-03
9	Mn	1.500E-06
10	Fe	1.700E-05
12	Co	3.800E-11
13	Ni	3.300E-11
14	Cu	5.560E-07
15	Zn	1.500E-08
17	Cd	8.900E-12
19	Ba	1.400E-11
52	Cl	1.910E-04
53	NO3	1.800E-04
54	SO4	1.000E-04
57	PO4	3.700E-07

RESULTS

NO. OF ITERATIONS	50

PH	8.000E+00
IONIC STRENGTH	5.605E-03
CHARGE RATIO	4.427E+00
CHARGE DIFFERENCE	+3.981E-03
ZED-FA	-2.309E-03
RATIO-FA	1.879E+00
WATER VOLUMES	
FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	3.220E-03
FRACTION SOLUTION	0.997E+00
CARBONATE ALKALINITY	+6.260E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES		S	DHA	DFA	HA	FA
3	Na	0.994	0.000	0.006	0.000	0.000
4	Mg	0.977	0.000	0.011	0.000	0.012
5	Al	0.973	0.000	0.000	0.000	0.027
6	K	0.994	0.000	0.006	0.000	0.000
7	Ca	0.977	0.000	0.011	0.000	0.012
9	Mn	0.924	0.000	0.010	0.000	0.066
10	Fe	0.379	0.000	0.003	0.000	0.618
12	Co	0.954	0.000	0.008	0.000	0.039
13	Ni	0.753	0.000	0.005	0.000	0.242
14	Cu	0.020	0.000	0.000	0.000	0.980
15	Zn	0.381	0.000	0.004	0.000	0.615
17	Cd	0.752	0.000	0.008	0.000	0.239
19	Ba	0.985	0.000	0.011	0.000	0.004
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

SOURCE FILE	Copper IC50 (RR5 days)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00

INPUT DATA

TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	2.120E-02
PCO2	3.500E-04

MASTER SPECIES		TOTAL CONC
3	Na	3.900E-04
4	Mg	2.800E-04
5	Al	3.700E-07
6	K	2.900E-06
7	Ca	2.170E-03
9	Mn	1.500E-06
10	Fe	1.700E-05
12	Co	3.800E-11
13	Ni	3.300E-11
14	Cu	2.220E-07
15	Zn	1.500E-08
17	Cd	8.900E-12
19	Ba	1.400E-11
52	Cl	1.910E-04
53	NO3	1.800E-04
54	SO4	1.000E-04
57	PO4	3.700E-07

RESULTS

NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.619E-03
CHARGE RATIO	4.440E+00
CHARGE DIFFERENCE	+3.995E-03
ZED-FA	-2.299E-03

RATIO-FA	1.875E+00
WATER VOLUMES	
FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	2.790E-03
FRACTION SOLUTION	0.997E+00

CARBONATE ALKALINITY +6.264E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES		S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.980	0.000	0.010	0.000	0.010
5	Al	0.978	0.000	0.000	0.000	0.022
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.980	0.000	0.010	0.000	0.010
9	Mn	0.935	0.000	0.009	0.000	0.056
10	Fe	0.428	0.000	0.003	0.000	0.569
12	Co	0.961	0.000	0.007	0.000	0.033
13	Ni	0.788	0.000	0.005	0.000	0.208
14	Cu	0.024	0.000	0.000	0.000	0.976
15	Zn	0.430	0.000	0.003	0.000	0.567
17	Cd	0.786	0.000	0.008	0.000	0.207
19	Ba	0.987	0.000	0.010	0.000	0.004
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

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SOURCE FILE	IC50 (RR 10 days)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00
INPUT DATA	
TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	1.960E-02
PCO2	3.500E-04

MASTER SPECIES		TOTAL CONC
3	Na	3.900E-04
4	Mg	2.800E-04
5	Al	3.700E-07
6	K	2.900E-06
7	Ca	2.170E-03
9	Mn	1.500E-06
10	Fe	1.700E-05
12	Co	3.800E-11
13	Ni	3.300E-11
14	Cu	1.700E-08
15	Zn	1.500E-08
17	Cd	8.900E-12
19	Ba	1.400E-11
52	Cl	1.910E-04
53	NO3	1.800E-04
54	SO4	1.000E-04
57	PO4	3.700E-07

RESULTS	
NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.626E-03
CHARGE RATIO	4.446E+00
CHARGE DIFFERENCE	+4.003E-03
ZED-FA	-2.293E-03
RATIO-FA	1.873E+00
WATER VOLUMES	
FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	2.576E-03

FRACTION SOLUTION 0.997E+00
CARBONATE ALKALINITY +6.265E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES		S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.982	0.000	0.009	0.000	0.009
5	Al	0.980	0.000	0.000	0.000	0.020
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.982	0.000	0.009	0.000	0.009
9	Mn	0.940	0.000	0.008	0.000	0.052
10	Fe	0.453	0.000	0.003	0.000	0.543
12	Co	0.964	0.000	0.006	0.000	0.030
13	Ni	0.804	0.000	0.004	0.000	0.192
14	Cu	0.027	0.000	0.000	0.000	0.973
15	Zn	0.455	0.000	0.003	0.000	0.541
17	Cd	0.802	0.000	0.007	0.000	0.191
19	Ba	0.988	0.000	0.009	0.000	0.003
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

SOURCE FILE Nickel IC50 (0 days)
DATABASE water10
PH FIXED
PRECISION % 1.000E-02
STARTING PH 8.000E+00

INPUT DATA
TEMPK 2.930E+02
TOT HA 0.000E+00
TOT FA 2.440E-02
PCO2 3.500E-04

MASTER SPECIES		TOTAL CONC
3	Na	3.900E-04
4	Mg	2.800E-04
5	Al	3.700E-07
6	K	2.900E-06
7	Ca	2.170E-03
9	Mn	1.500E-06
10	Fe	1.700E-05
12	Co	3.800E-11
13	Ni	1.280E-06
14	Cu	1.600E-11
15	Zn	1.500E-08
17	Cd	8.900E-12
19	Ba	1.400E-11
52	Cl	1.910E-04
53	NO3	1.800E-04
54	SO4	1.000E-04
57	PO4	3.700E-07

RESULTS
NO. OF ITERATIONS 50
PH 8.000E+00
IONIC STRENGTH 5.606E-03
CHARGE RATIO 4.427E+00
CHARGE DIFFERENCE +3.982E-03
ZED-FA -2.307E-03
RATIO-FA 1.879E+00
WATER VOLUMES
FRACTION HA-DDL 0.000E+00
FRACTION FA-DDL 3.219E-03
FRACTION SOLUTION 0.997E+00
ARBONATE ALKALINITY +6.260E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES	S	DHA	DFA	HA	FA

3	Na	0.994	0.000	0.006	0.000	0.000
4	Mg	0.977	0.000	0.011	0.000	0.012
5	Al	0.972	0.000	0.000	0.000	0.028
6	K	0.994	0.000	0.006	0.000	0.000
7	Ca	0.977	0.000	0.011	0.000	0.012
9	Mn	0.924	0.000	0.010	0.000	0.066
10	Fe	0.371	0.000	0.003	0.000	0.626
12	Co	0.954	0.000	0.008	0.000	0.039
13	Ni	0.747	0.000	0.005	0.000	0.248
14	Cu	0.019	0.000	0.000	0.000	0.981
15	Zn	0.373	0.000	0.004	0.000	0.623
17	Cd	0.748	0.000	0.008	0.000	0.244
19	Ba	0.985	0.000	0.011	0.000	0.004
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

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SOURCE FILE	Nickel IC50 (5 days)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00
INPUT DATA	
TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	2.120E-02
PCO2	3.500E-04
MASTER SPECIES	TOTAL CONC
3 Na	3.900E-04
4 Mg	2.800E-04
5 Al	3.700E-07
6 K	2.900E-06
7 Ca	2.170E-03
9 Mn	1.500E-06
10 Fe	1.700E-05
12 Co	3.800E-11
13 Ni	5.380E-07
14 Cu	1.600E-11
15 Zn	1.500E-08
17 Cd	8.900E-12
19 Ba	1.400E-11
52 Cl	1.910E-04
53 NO3	1.800E-04
54 SO4	1.000E-04
57 PO4	3.700E-07
RESULTS	
NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.619E-03
CHARGE RATIO	4.440E+00
CHARGE DIFFERENCE	+3.996E-03
ZED-FA	-2.297E-03
RATIO-FA	1.875E+00
WATER VOLUMES	
FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	2.790E-03
FRACTION SOLUTION	0.997E+00
CARBONATE ALKALINITY	+6.264E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES		S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.980	0.000	0.010	0.000	0.010
5	Al	0.978	0.000	0.000	0.000	0.022
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.980	0.000	0.010	0.000	0.010

9	Mn	0.935	0.000	0.009	0.000	0.056
10	Fe	0.424	0.000	0.003	0.000	0.573
12	Co	0.960	0.000	0.007	0.000	0.033
13	Ni	0.785	0.000	0.005	0.000	0.210
14	Cu	0.024	0.000	0.000	0.000	0.976
15	Zn	0.426	0.000	0.003	0.000	0.570
17	Cd	0.784	0.000	0.008	0.000	0.209
19	Ba	0.987	0.000	0.010	0.000	0.003
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	0.999	0.000	0.001	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000

SOURCE FILE	IC50 (RR10 days-nickel)
DATABASE	water10
PH	FIXED
PRECISION %	1.000E-02
STARTING PH	8.000E+00
INPUT DATA	
TEMPK	2.930E+02
TOT HA	0.000E+00
TOT FA	1.900E-02
PCO2	3.500E-04
MASTER SPECIES	TOTAL CONC
3 Na	3.900E-04
4 Mg	2.800E-04
5 Al	3.700E-07
6 K	2.900E-06
7 Ca	2.170E-03
9 Mn	1.500E-06
10 Fe	1.700E-05
12 Co	3.800E-11
13 Ni	3.500E-07
14 Cu	1.600E-11
15 Zn	1.500E-08
17 Cd	8.900E-12
19 Ba	1.400E-11
52 Cl	1.910E-04
53 NO3	1.800E-04
54 SO4	1.000E-04
57 PO4	3.700E-07
RESULTS	
NO. OF ITERATIONS	50
PH	8.000E+00
IONIC STRENGTH	5.629E-03
CHARGE RATIO	4.449E+00
CHARGE DIFFERENCE	+4.006E-03
ZED-FA	-2.290E-03
RATIO-FA	1.871E+00
WATER VOLUMES	
FRACTION HA-DDL	0.000E+00
FRACTION FA-DDL	2.495E-03
FRACTION SOLUTION	0.998E+00
CARBONATE ALKALINITY	+6.266E-04

FRACTIONAL DISTRIBUTIONS OF MASTER SPECIES

MASTER SPECIES		S	DHA	DFA	HA	FA
3	Na	0.995	0.000	0.005	0.000	0.000
4	Mg	0.982	0.000	0.009	0.000	0.009
5	Al	0.981	0.000	0.000	0.000	0.019
6	K	0.995	0.000	0.005	0.000	0.000
7	Ca	0.982	0.000	0.009	0.000	0.009
9	Mn	0.943	0.000	0.008	0.000	0.050
10	Fe	0.467	0.000	0.003	0.000	0.530
12	Co	0.965	0.000	0.006	0.000	0.029
13	Ni	0.812	0.000	0.004	0.000	0.184
14	Cu	0.028	0.000	0.000	0.000	0.972

15	Zn	0.469	0.000	0.003	0.000	0.528
17	Cd	0.809	0.000	0.007	0.000	0.184
19	Ba	0.988	0.000	0.009	0.000	0.003
52	Cl	1.000	0.000	0.000	0.000	0.000
53	NO3	1.000	0.000	0.000	0.000	0.000
54	SO4	1.000	0.000	0.000	0.000	0.000
57	PO4	0.999	0.000	0.001	0.000	0.000