EFFECT OF A METAL MIXTURE (Cu, Zn, Pb AND Ni) ON THE BIOAVAILABILITY AND BIOACCUMULATION OF CADMIUM IN NATURAL SYSTEMS

BY

A. ROBIN STEWART

A Thesis
Submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Botany
University of Manitoba
Winnipeg, Manitoba

National Library of Canada
Acquisitions and Bibliographic Services
395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque nationale du Canada
Acquisitions et services bibliographiques
395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

Our file Notre référence

0-612-32461-3

Canada

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

EFFECT OF A METAL MIXTURE (Cu,Zn,Pb AND Ni) ON THE BIOAVAILABILITY AND BIOACCUMULATION OF CADMIUM IN NATURAL SYSTEMS

BY

A. ROBIN STEWART

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

DOCTOR OF PHILOSOPHY

ABSTRACT

Molluscs were evaluated as biomonitoring tools for the Canadian mining industry. Bivalve molluscs were found to be appropriate biomonitors of metals in the aquatic environment, particularly for characterizing spatial and temporal trends in metal contamination. Further research was recommended to develop molluscs as biomonitors of tissue-residue based effects of metals.

The effects of a metal mixture (Cu, Zn, Pb and Ni) on Cd bioavailability and accumulation by a freshwater unionid mussel, *Pyganodon grandis*, and the isoetid macrophyte, *Eriocaulon septangulare*, were examined *in situ* at the Experimental Lakes Area, Ontario, Canada. A limnocorral experiment was conducted during the summer of 1992, in which Cd was added alone to the water column in treatment 1 and with the metal mixture in treatments 2, 3 and 4 to raise background sediment Cd concentrations by 7 times. Copper, Zn, Pb and Ni were added to treatments 2, 3 and 4 to raise sediment concentrations by 3, 4 and 7 times, respectively. Treatments with the metal mixture had longer residence times for Cd in the water column than the treatment with Cd alone. Cadmium accumulation in mussels was significantly reduced in treatments with the highest concentration of the metal mixture compared to treatments with the lowest concentration of the metal mixture or with Cd alone. Tissue metallothionein levels were highest in the kidney and tended to decrease in treatments with increasing metal addition. The effect of competition on the partitioning of Cd in the water column appeared to be a less important phenomenon than competition at binding sites on the mussels in determining Cd uptake by the mussels.

In the summer of 1995, littoral sediments were spiked with Cd alone and with the metal mixture at three increasing concentration levels (2, 4 and 6 times background). *Eriocaulon septangulare* was planted in the spiked sediment and placed at 0.5 m water depth in the littoral zone. The distribution of Cd among sediment fractions (easily-reducible, reducible and organic), in porewater, and in macrophytes was determined every second week for 10 weeks. The metal mixture had a significant affect on the

distribution of Cd among geochemical fractions in the sediments after 2 and 8 weeks, but not after 10 weeks. At the highest concentration of the metal mixture, Cd shifted from the easily-reducible (Mn-oxide) fraction which is considered more "bioavailable" onto the less bioavailable reducible (Fe-oxide) and organic fractions. The highest Cd concentrations were found in the shoots of plants in the treatment with Cd alone and the treatment with the highest concentration of the metal mixture.

ACKNOWLEDGEMENTS

My supervisor and mentor, Dr. Diane Malley, provided invaluable experience, support and advice. Committee members, Drs. Tee Boon Goh, Gordon Robinson and Sam Badour were generous with their time and provided constructive and thought-provoking criticism throughout this study. Botany Department Head, Dr. David Punter, guided me through the maze of graduate school administration and I am grateful for his help.

Field support for the setting up, sampling and removal of two labor intensive experiments came from Denise Tenkula, Morris Holoka, Bob Herzog, Eva Schindler, Neil Fisher, Marcia Kyle and John Embry. I cannot thank these people enough.

Numerous scientists and graduate students offered helpful scientific advice including Drs. Ray Hesslein, David Huebert, Michael Turner, Vince St. Louis, Andrew Heyes, Wayne Fairchild and Mr. Robert Currie. Metal analyses were carried out with the help of Robert Hunt, Carolyn Ranson, Sheri Friesen and Jack Boughen who cheerfully answered countless questions. Water chemistry analyses were performed by Eva Schindler, Ron Schade and support staff. Statistical advice was provided with great patience by Susan Kasian and Dr. Bridgette deMarch.

This project was supported by the DFO Green Plan Toxic Chemicals Program and a University of Manitoba Fellowship. I am grateful to the Fish Habitat Research Division for providing office space and equipment and to the Diver for Science Program operated by Bruce Townsend that enable me to gain the much needed SCUBA certification and first introduced me to Dr. Diane Malley.

Finally, to my parents, Bev and Fraser, and siblings, Pam and Chris, thanks for believing in me.

DEDICATION

For my husband, companion and best friend,

Robert Herzog.

Thanks for your continuous emotional, spiritual and financial support over the last 6 years. I gratefully acknowledge the sacrifices you made so I could achieve my career goals. Your patience was truly remarkable in many ways, but particularly after I asked you to readjust the 3,000 lb. of sand-bags you had so carefully placed underwater, "without disturbing the bottom sediment".

You bring joy to my life and color my rainbow
with red, yellow and blue.

FOREWARD

This doctoral thesis is organized as a series of manuscripts. The first manuscript forms part of a report prepared for the Canadian Centre for Mineral and Energy Technology (CANMET), Natural Resources Canada (NRC). The complete report has been published by the NRC, and the section found here will be revised and submitted as a review paper. The second manuscript has been submitted to the Canadian Journal of Fisheries and Aquatic Sciences and is currently under review. The final manuscript will be submitted to the journal, Environmental Toxicology and Chemistry. These manuscripts are coordinated by an overall introduction and discussion. A glossary of terms used in the thesis is provided at the start of the main body of the thesis. A combined list of references is found following the three papers and is formatted for the Canadian Journal of Fisheries and Aquatic Sciences.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY

Acute - Having a sudden onset, lasting a short time. Of a stimulus, severe enough to induce a response rapidly. Can be used to define either the exposure or the response to an exposure (effect). For clarity, the length of the exposure (short, medium, or long) and the nature of the effect endpoint (lethal or sublethal) should be specified. The duration of an acute aquatic toxicity test is generally 4 d or less and mortality is the response measured.

Antagonism - action between two or more substances in which one interferes with the action of the other so that there is less effect of a substance when in the presence of the other than if it were present alone.

Apparent partitioning coefficient - A measure of the affinity of a contaminant for particles calculated as the ratio of the contaminant concentration on particles to the dissolved contaminant concentration. The term "apparent" refers to the fact that the system is not in equilibrium.

Bioaccumulation - general term describing a process in which chemicals are taken up by aquatic organisms directly from water as well as through exposure through other routes, such as consumption of food and sediment containing the chemicals. It includes a net retention of a contaminant by an organism over time, that is the influx of a contaminant exceeds its efflux. Bioaccumulation may not be associated with an increase in an organism's contaminant concentration, which may be caused by a loss in body weight.

Bioaccumulation factor (BAF) - A value that is the ratio of tissue chemical residue to chemical concentration in an external environmental phase (i.e., sediment or food). The BAF is measured at steady state in situations where organisms are exposed to multiple sources (i.e., water, sediment, food), unless noted otherwise.

Bioavailability - the portion of a contaminant in the environment whose presence in or mobilization from water, sediment, or diet gives rise to measurable accumulation by an organism.

Bioconcentration - a process by which there is a net accumulation of a chemical directly from water into aquatic organisms resulting from simultaneous uptake (e.g., by gill or epithelial tissue) and elimination.

Bioconcentration factor (BCF) - unit-less term describing the degree to which a chemical can be concentrated in the tissues of an organism in the aquatic environment as a result of exposure to water-borne chemical. The BCF is calculated at steady-state as the ratio of the tissue metal concentration to the water metal concentration.

Biomagnification - the accumulation of a substance from food organisms leading to a higher concentration in organisms of a higher trophic level.

Biomarker - a biological response to the exposure to an environmental chemical. This response at the below-individual level, is not necessarily detected at the whole organism level.

Body Burden (=Content)- the total amount of contaminant found in an organism. Usually determined by multiplying the body/tissue metal concentration by the weight of the body/tissues.

Chronic - Involving a stimulus that is lingering or continues for a long time; often signifies periods from several weeks to years, depending on the reproductive life cycle of the aquatic species. Can be used to define either the exposure or the response to an exposure (effect). For clarity the length of response the nature of the effect endpoint should be specific.

Content (=Body burden)- the total amount of contaminant found in an organism. Usually determined by multiplying the body/tissue metal concentration by the weight of the body/tissues.

Effluent - outflow; liquid waste which is discharged into a water (e.g. river) system

Endemic - species is restricted to a single geographical area.

Exposure - the amount of contaminant an organism is subjected to in the environment. Exposure is a function of contaminant concentration and bioavailability.

Equilibrium partitioning (EqP) - An approach for estimating the fate of chemicals (primarily organics) in the aquatic environment that is based on the assumption that a steady-state can be achieved, and usually is achieved, between the activity of chemicals (usually approximated as a concentration) in the various component phases - water, sediment, organisms.

Dioecious - an individual possessing either a male or female reproductive system; the reproductive systems are contained in separate individuals.

Dose - is the concentration of a contaminant in an organism's tissues. Contaminant toxicity is derived from Dose-Response relationships.

Gonochoristic - sexes are separate; males and females in the same population. Reproduction is accomplished through cross-fertilization.

Hermaphroditic - an individual that has both male and female reproductive organs.

Isoetid - aquatic plants that are characterized by a very short stem and a rosette of stiff leaves.

Iteroparous - reproducing repeatedly at multiple times during the life of an organism before it dies.

K_d - partition coefficient of a trace element on particles.

LC_{50} - the concentration of a toxin which is fatal for 50 % of the test organisms, used in tests of aquatic organisms in place of LD50.

LD_{50} - the dose or amount of a toxin which kills half of a test population of a particular species; it can differ among species

Lacunae - multicellular cavities in plants. Extensive lacunae system in isoetids store photosynthetic gases and allow for easy transport between the roots and shoots.

Metallothionein (MT) - cysteine-rich, heat-stable, low-molecular weight protein which chelates metals such as Cd, Cu and Zn. Functions attributed to MT include detoxification, and regulation of these metals.

Metal mixture - combination of two or more metals.

Metal species - the molecular representation of a physiochemical form of an element.

Monoecious - having both the male and female reproductive systems in one individual.

Ovoviviparous - females that lay eggs which hatch within the body of the female and are released as free-living offspring.

Pandemic species - species occurs throughout the whole country or world.

Partitioning coefficient - A measure of the affinity of a contaminant for particles calculated as the ratio of the contaminant concentration on particles to the dissolved contaminant concentration.

Pseudofeces - masses of mucous-bound particles rejected from the labial palps of bivalve molluscs before ingestion and released into the mantle cavity to be carried out the siphon on water currents induced by valve clapping (i.e., rapid valve adduction).

Semelparous - breeding only once during life history; one mass reproductive effort.

Sentinel organism - organisms that are used to monitor contaminants in the environment through the accumulation of contaminants in their tissues.

Steady-state - when used in the context of toxicokinetic studies, refers to the condition in which organism uptake and excretion rates are balanced and further net bioaccumulation does not occur.

Sub-lethal - Below the concentration that directly causes death. Exposure to sublethal concentrations of a material may produce less obvious effects on behavior, biochemical, and/or physiological function, and histology of organisms.

Synergism - the effect of two chemicals which, in combination, act more strongly than either alone.

Tolerance - an individual organism shows fewer or no adverse effects to a substance after repeated exposures.

Toxicity - capacity of a chemical to cause injury to a living organism. This is usually defined with reference to:

a. the species and its life stage,

b. the dose of the chemical,

c. distribution of dose in time (acute dose, chronic dose),

d. type and severity of injury (lethal response, sub-lethal response e.g., immunotoxicity, genotoxicity, cytoxicity, avoidance),

e. time needed to produce injury (acute, chronic).

Zoogeographic Region - Geographical region identified by a group of species that do not occur anywhere else.

CHAPTER 1. INTRODUCTION

Rationale for thesis

Over the past decade, although there has been increasing attention to persistent organic contaminants, greenhouse gases and global warming, toxic heavy metals in the aquatic environment remain an enduring threat. Continued introduction of metals into the aquatic environment by industrial processes, domestic practices and mining have ensured that the levels of contamination continue to be a problem (Nriagu 1990). Anthropogenic emissions of Pb, Cd, Va, and Zn exceed the fluxes from natural sources such as volcanoes, forest fires and soil weathering by 28-, 6-, 3- and 3-fold, respectively (Nriagu 1990). The world-wide anthropogenic input of trace metals into aquatic ecosystems quantified as of 1988 were 9.1, 112, 237, 114, and 138 (in thousands of tonnes per year) for Cd, Cu, Zn, Pb and Ni, respectively (Nriagu and Pacyna 1988). The broad distribution patterns of metals released into the environment means that they cannot be efficiently recovered or recycled.

Of the metals released into the aquatic environment, Cd is thought to pose one of the most significant threats. Cadmium is bioaccumulated, serves no essential physiological function, and is highly toxic at low concentrations (Nriagu 1990; Borgmann et al. 1991; Lawrence and Holoka 1991). The concern over Cd in the aquatic environment has led to a whole-lake ecosystem study of the effects of Cd added at low environmental concentrations (<0.2 $\mu g\ L^{-1}$ (1987 Canadian Water Quality Guideline) from 1987-1991) (Malley 1996).

Upon entering aquatic systems, metals move downward through the water column towards the sediment during which time they can be accumulated by pelagic organisms (fish, zooplankton and phytoplankton). Ultimately, metals reach the sediment where they accumulate (Livett 1988). On a weight per square meter basis, the uppermost-surficial sediments constitute the largest trace metal reservoir in aquatic systems (see metal mass balance calculations in St-Cyr et al. 1994). Once accumulated in sediments, the metals continue to pose a threat to aquatic life due to re-suspension into the water column from

geochemical re-cycling (Santchi et al. 1986; Wagemann et al. 1994; Campbell and Tessier 1996), accumulation in benthic fauna that feed on sediments (Hare et al. 1994) and through food chain transfer, including organo-elements such as Hg and Se (Lemly 1993; Luoma et al. 1992). As well, the world wide dredging of contaminated sediments in harbors and waterways release metals back into the water column (ICCS 1997).

The development of regulatory protocols for metals in water and sediment has changed over the past 20 years with new technologies and improved understanding of those factors that influence the fate and toxicity of metals. During the mid to late 80's the emphasis was placed on the development of guidelines for metals in water and sediment for the protection of aquatic life (CCME 1987). These criteria levels were based on acute and chronic laboratory toxicity data for the most sensitive organisms and could be varied along a sliding scale for hardness which adjusts for the influence of pH and water hardness on metal toxicity, i.e., the tendency for increased toxicity at low pH (Campbell and Stokes 1985). However, criteria levels did not take into consideration other important factors that influenced the "potency" of the metals under varying geochemical and biological conditions. Furthermore, the guidelines were based on single metals and did not account for the potential interactions among metals that may change the toxicity of a single metal.

Metals are not found individually in aquatic systems, but instead occur in complex mixtures composed of metals and organic contaminants. A review for the European Inland Fisheries Advisory Commission (EIFAC) recommends that joint effects models (based on additive, less-than additive, and synergistic effects) predict joint acute-lethal effects of toxicants in sewage and industrial effluents well enough to be applied in the development of water quality criteria (EIFAC 1987). However, it was also reported that there is an immediate need for information in the laboratory or field on long-term lethal and sub-lethal joint effects and their relationship with accumulation patterns of the toxicants in question.

A relatively new approach to assessing and regulating toxic metals is the utilization of field-based "biomonitoring organisms" (Phillips and Rainbow 1993). Absolute concentrations and toxicity in organisms from different trophic levels are monitored to determine spatial and temporal trends in contamination in the field as well

as compliance by industry to regulatory guidelines. The biomonitoring approach is versatile in that it can be site-specific, can be used to assess effects of metals as well as basic mechanisms of metal bioavailability and bioaccumulation and the influence of geochemical and biological factors. Monitoring organisms are not necessarily the most sensitive species, but their interactions with metals allow for a better understanding of metal dynamics in the aquatic environment (Phillips and Rainbow 1993). An important component of biomonitoring is a thorough understanding of the mechanisms of metal uptake and toxicity in the target organisms, which comes through combined field and laboratory study.

Objectives

This thesis has two main objectives. The first objective is an evaluation of molluscs as biomonitoring tools for the mining industry and is based on a review of scientific primary publications and grey literature. All aspects related to the use of molluscs to monitor metals in the aquatic environment are reviewed, knowledge gaps identified, and a final evaluation of the usefulness of molluscs based on a clearly defined set of criteria is provided. The second objective of the thesis is to describe experiments in which metal interactions were examined in the field using two biomonitoring organisms. Specifically, this objective was to examine the effect of a metal mixture Cu, Zn, Pb and Ni on the distribution of Cd experimentally added to water and sediment *in situ* and on Cd accumulation in two benthic organisms. Field studies were designed to answer two primary questions: 1) Are there differences in the partitioning of Cd in the environment in the presence of a metal mixture compared to Cd added alone and do the differences depend on the concentration of the mixture (for the range tested)?; and 2) Are there differences in the accumulation of Cd by the benthic organisms in the presence of the metal mixture compared to Cd alone, and if so, can they be explained by the effect of the metal mixture on the partitioning of Cd in the environment? Two experiments were designed specifically to examine metal interactions in the water column and in sediments, respectively. The first experiment involved the introduction of Cd and a metal mixture to limnocorrals allowing a portion of the water column to be isolated from the lake and spiked with metals. It utilized the freshwater mussel *Pyganodon grandis*, a filter-feeder,

which accumulates metals from the water column (directly from water or from food particulates). In the second experiment, involving the spiking of littoral sediments, the macrophyte *Eriocaùlon septangulàre* was chosen since it is known to accumulate metals predominantly from the sediment.

The metals chosen for the mixture (Cu, Zn, Pb and Ni) are commonly released with Cd during industrial processes (Scheider et al. 1981; Jeffries and Snyder 1981; Nriagu 1990; AQUAMIN 1996) and should provide a realistic assessment of the types of interactions that might take place under natural conditions. The design of both experiments did not allow for specific investigation of the nature of interactions among metals, rather these studies were meant to determine if the presence of metals in mixtures is an important modifying factor in metal bioaccumulation.

CHAPTER 2. DESCRIPTION OF *ERIOCAÙLON SEPTANGULÀRE*

Eriocaùlon septangulàre With. is a freshwater, aquatic vascular plant of the Class Monocotyledonae, Order Eriocaulales, Family Eriocaulàceae (Robinson and Fernald 1909). The name *Erio-* "wool" and *caulon-* "stem" refer to a stem-filled with wooly parenchyma and *sept-* "seven" and *angulare-* "angled" refer to its 4-7 ridges found on its stem. Commonly known as duckgrass, *E. septangulàre* is a rooted aquatic macrophyte with vegetative structures that emerge above the water surface. It is classified as a rhizophyte (rooted in sediment) and helophyte (emergent vegetative structures) according to Hutchinson (1975).

Eriocaùlon septangulàre has an isoetoid growth form characterized by a very short stem and a rosette of stiff leaves. It is less than 10 cm tall, with long and slender leaves (2-8 cm) that taper into a point (Robinson and Fernald 1909). The leaves are round in cross-section and possess cross-veins. The roots are white and fibrous with slightly constricting cross markings. Its flowers are button-like heads on tips of slender stalks with bracts at the base.

Eriocaùlon septangulàre has a temperate distribution occurring throughout the eastern provinces of Canada to the Prairies, the northeastern United States, as well as western Ireland and Scotland (Robinson and Fernald 1909). *Eriocaùlon septangulàre* appears to be relatively tolerant of low pH, with high abundances in the acidic oligotrophic lakes of the Adirondack region of New York (Roberts et al. 1985).

Like other isoetids, *E. septangulàre* is generally found growing in shallow (< 2m), exposed sandy areas of lakes or streams as dense multispecies mats or as scattered individuals and clumps (Moeller 1975; Hitchin et al. 1984). It has been found in dense mats devoid of other species or in mats shared with subdominant species such as *Myriophyllum tenellum* Bigellow, *Utricularia resupinata* Greene, *Elatine minima* (Nuttall) Fisher & Meyer, *Fontinalis antipyretica* L. ap. Hedw. and *Isoetes* spp (France and Stokes 1988). *Eriocaùlon septangulàre*-dominated communities have been most often located proximal to the mouths of inflows (France and Stokes 1988). *Eriocaùlon*

septangulàre is associated with many epiphytic macroinvertebrates, particularly the amphipod *Hyallela azteca* (Howell 1990; France 1987). Isoetid habitats are typically low in nutrients and inorganic carbon and receive moderately high amounts of light. Factors that may influence the distribution patterns of isoetids include substratum, light, mode of spread and disturbance (Boston 1986; Wilson and Keddy 1985).

Eriocaùlon septangulàre is a perennial with long-lived evergreen leaves, although it does experience small seasonal changes in biomass. It utilizes sediment CO_2 (>89-99%) through a specialized gas exchange system including morphological adaptations to their leaves and roots (thick leaves and cuticle; chloroplasts lining the lacunae; continuous lacunae connecting the roots and leaves) and an oxygen-release mechanism from their roots (Boston et al. 1987). Unlike *Isoetes* spp., *E. septangulàre* does not appear to utilize bicarbonate and therefore is a C_3 - plant and not a CAM plant (CAM - crassulacean acid metabolism) (Boston et al. 1987).

CHAPTER 3. EVALUATION OF MOLLUSCS AS BIOMONITORING TOOLS FOR THE CANADIAN MINING INDUSTRY

3.1 Summary

The Aquatic Effects Technology Evaluation Program, AETE has been established to assist the Canadian mining industry in meeting its environmental effects monitoring and related requirements, in as cost-effective a manner as possible. The program is coordinated by the Canadian Center for Mineral and Energy Technology (CANMET). The present report is a technical evaluation of molluscs as biomonitoring tools for the mining industry.

Molluscs are a diverse taxonomic group that include bivalves and gastropods and are found widely distributed throughout Canada. Molluscs have been widely studied in both the laboratory and the field in order to evaluate their ability to serve as biomonitors of metals in the aquatic environment. The present evaluation of molluscs as biomonitors is based on published studies conducted in the laboratory and field on mining contaminants of concern listed in the AQUAMIN Report (AQUAMIN, 1996). Published field studies and reports of studies carried out by individual mining companies and the AETE program were consulted. Criteria for a good biomonitor and the work requirements of the contract were used as guidelines for the evaluation process. The following are the conclusions of this evaluation based on individual criteria.

1. **Biomonitors should be relatively non-mobile in order that their exposure be representative of the study area.** Molluscs are relatively sedentary, although some species (i.e. unionids) may migrate short distances (meters) within their habitat. Bivalve molluscs are amenable to transplanting and caging. The sedentary nature and ease of caging and retrieval of molluscs are advantages for site-specific monitoring not possessed by most fish.

2. **Biomonitors should be abundant, widely distributed, easy to identify and sample at all times of the year.** Molluscs are widely distributed across Canada and can be identified with limited taxonomic expertise. The limited information on the conservation status of molluscs suggests that most unionid bivalve species are currently stable in Canada.
3. **Biomonitors should be large enough in body size to provide sufficient tissue for analysis.** Individual unionid bivalves are large enough to provide sufficient tissue for metal analyses. Pooling of individuals of the smaller sphaeriids and most gastropods would be required for tissue metal analysis.
4. **Biomonitors should be hardy, tolerating wide ranges of contaminant concentration and physiochemical variables.** Molluscs are relatively hardy and tolerate wide ranges of metal concentrations, but are limited by pH below 4.7 and [Ca] below 2 mg L^{-1}.
5. **Biomonitors should be strong accumulators of metals, with a simple correlation between metal concentrations in mollusc tissue and average ambient metal concentrations.** Molluscs strongly accumulate metals (Cd, Cu, Zn, Pb, Ni, Hg, As, Ag and Cr). Field studies suggest that the relationship between mollusc tissue metal concentrations and ambient metal concentrations is influenced by a number of biological, physical and chemical parameters that need to be taken into account. Ultimately, the relationship is metal specific and depends on the availability of the metal from the dissolved and particulate phase.

Having evaluated molluscs on the above criteria these are the following recommendations made to Natural Resources Canada and the Canadian mining industry:

1. Molluscs can be used as indicators of exposure to metals such as Cd, Cu, Zn, Pb, Ni, Hg, As, Cr, and Ag. Metal concentrations in bivalve molluscs could be used to:
    a) confirm changes in biologically-relevant metal concentrations in the natural environment resulting from mining activities.

b) monitor long-term site-specific spatial and temporal trends in biologically-relevant metal concentrations by increases and decreases in tissue concentrations of indigenous or transplanted populations.

c) determine the effectiveness of remedial measures through the use of transplanted and indigenous molluscs.

2. Molluscs are not stand-alone tools. Numerous abiotic and biotic measurements must be made in conjunction with metal concentrations in molluscs to interpret effectively the results of field studies.

3. Metal-induced effects in molluscs such as changes in growth, or MT concentrations are not well established. These responses could be measured as part of the monitoring program, but their results should be used with caution as measures of effect until their role has been validated in field studies using mining contaminants.

4. Molluscs could be used in the first steps of a monitoring program in order to assess the extent of contamination in the aquatic environment. In more detailed information stages, molluscs may be used to investigate site-specific sources of bioavailable metals, improvements to waste-water treatment and effectiveness of remedial actions.

5. For comprehensive biomonitoring, molluscs can be used in conjunction with several other organisms (e.g. invertebrates, fish, plants) to monitor metals and their effects in the aquatic environment. In this way, information on the "biological consequences" of mining operations can be obtained that is not available from monitoring sediment and water only.

3.2. Introduction

The Aquatic Effects Technology Evaluation (AETE) program was established to review appropriate technologies for assessing the impacts of mine effluents on the aquatic environment. AETE is a cooperative program among the Canadian mining industry, several federal government departments and a number of provincial governments. It is coordinated by the Canadian Center for Mineral and Energy Technology (CANMET) of Natural Resources Canada. One mandate of the AETE is to do a field and technical evaluation of molluscs as a biomonitoring tool for the mining industry in Canada. This report represents the technical component of the evaluation.

Mining, smelting and refining of minerals and metals are associated with a number of environmental concerns (The State of Canada's Environment, 1996). Acid-mine drainage and sulfur dioxide, particulate matter, and heavy metal emissions are considered to pose the largest problems. In order to adequately assess the impacts resulting from present mines (active and abandoned) and estimate the potential impacts from future developments, effective environmental monitoring programs are needed.

The Canadian government's Metal Mine Liquid Effluent Regulations (MMLER) require the monitoring of the quality of liquid effluent, but not that of receiving waters. Monitoring of the receiving environment is usually performed as a result of an environmental assessment or through a Provincial and Territorial program (AQUAMIN, 1996). The development of site-specific environmental effects monitoring programs by mine operators was one of the key recommendations in the Assessment of the Aquatic Effects of Mining (AQUAMIN) in Canada report (AQUAMIN, 1996). The AQUAMIN working group, composed of scientists and industry representatives across Canada, recommended that the monitoring programs be part of a national monitoring program with consistent study objectives, approaches and methods.

The contaminants that might be included in a national monitoring program for mining would consist of those regulated under the current MMLER, i.e., As, Cu, Pb, Ni, Zn, and ^{226}Ra (AQUAMIN, 1996). In addition, cyanide will also be added to the list of MMLER contaminants on the recommendation of the AQUAMIN working groups.

Additional contaminants not listed under the MMLER but recommended for monitoring by the AQUAMIN working groups include aluminium (Al), cadmium (Cd), calcium fluoride, iron (Fe), mercury (Hg), molybdenum (Mo), nitrogen compounds, and thiosalts. The effectiveness of molluscs as potential biomonitoring tools for the Canadian mining industry will be evaluated here in reference to these contaminants.

According to the AQUAMIN report, between 103 and 177 metal mines have been operated in Canada at any one time over the past 25 years. The majority of these mines discharge directly into the freshwater environment. Consequently, this review will focus on molluscs found in freshwater and their effectiveness in monitoring the prescribed contaminants.

3.3. Conceptual framework for molluscs as biomonitors

3.1.1 The biomonitoring concept

In its broadest sense, the term "biological monitoring" or "biomonitoring" can be defined as "the systematic use of biological responses to evaluate changes in the environment with the intent to use this information in a quality control program" (Rosenberg and Resh, 1993). There are several types of biomonitoring including surveillance and compliance compliance (Rosenberg and Resh, 1993). Surveillance usually includes surveys before and after a project is completed or before a toxicant is spilled and is used to determine if water management techniques are working or whether conservation measures are successful. Historical biomonitoring or long-term surveillance "can provide the evidence essential to the evaluation of apparent or emerging environmental problems" (Monitoring and Assessment Research Centre, 1985). Compliance monitoring is done either to ensure that regulatory requirements are being met or to maintain the course of long-term water quality goals (Rosenberg and Resh, 1993).

Biomonitoring programs measure various biological responses in order to evaluate changes in the environment including, (1) organism-level responses ranging from

biochemical to life-history changes (i.e. growth) and bioaccumulation, and (2) population or community level responses such as presence/absence or numerical predominance of indicator organism populations and species assemblages (Rosenberg and Resh, 1993). Ideally, a biomonitoring program should include both organism and population level biological responses using several resident taxa to assess different types of metal stress (Crawford and Luoma, 1993). This report will focus primarily on organism level bioaccumulative biomonitoring (i.e. in molluscs).

Bioaccumulative biomonitors, also called "sentinel organisms" (Goldberg et al. 1978), are used to measure contaminant concentrations in aquatic ecosystems through the accumulation of contaminants in their tissues. The use of sentinel organisms has many advantages over the analysis of abiotic components in that measurement of a pollutant in the organism signifies that the pollutant is (was) bioavailable and may be a threat not only to the organism itself but also to other parts of the food web and ecosystem (V.-Balogh, 1988). Sentinel organisms also provide a time-integrated measure of pollutant availability, in contrast to the instantaneous nature of pollutant concentrations measured in water. The primary difficulty faced when using sentinel organisms is separating changes resulting from trends in industrial exposure from changes in exposure resulting from environmental, biological or physico-chemical factors (Phillips and Rainbow, 1993).

In this context, biomonitoring essentially provides information on the variation in time and space of the concentrations of contaminants which are available to a selected biomonitor (Phillips and Rainbow, 1993). Thus, biomonitoring seeks to identify significant differences in contaminant bioavailabilities among samples from different sites, or among samples taken from a site at different times.

3.3.2 Criteria for a good biomonitor

Organisms are chosen as biomonitors because they possess characteristics that render them useful in monitoring programs (Phillips and Rainbow, 1993; Rosenberg and Resh, 1993):

1. Biomonitors should be relatively non-mobile, either through behavior (e.g. territorial behavior) or because they are sessile or sedentary, in order that their exposure be representative of the study area.
2. Biomonitors should be abundant in study areas, easy to identify and to sample at all times of the year.
3. Biomonitors should be large enough in body size to provide sufficient tissue for analysis of the contaminant of interest.
4. Biomonitors should be hardy, tolerating wide ranges of contaminant concentration and physiochemical variables such as salinity, temperature and acidity, thereby ensuring their ubiquity in the receiving environment and permitting the design of transplant experiments and laboratory studies of contaminant kinetics.
5. Biomonitors should be strong accumulators of the relevant contaminant, with a simple correlation between the metal concentration found in the tissues of the organism and the average ambient bioavailable contaminant concentration. This dose-response relationship should be independent of environmental factors and caging or respond in a predictable manner so as to control for these confounding factors.

Molluscs satisfy many of the above characteristics of a good biomonitor as summarized in Metcalfe-Smith (1994): wide distributions of closely related species maximize data comparability; sedentary habits maximize site-specific information; large, stable populations permit repeated sampling; specimens are readily sampled, handled, and identified; tolerances to many contaminants are high compared with other aquatic organisms; bioconcentration factors (BCFs) are high; and bivalves provide a measure of contaminant bioavailability near the entry level of the food chain. Interpreting metal accumulation in the tissues of aquatic biota, including molluscs, nevertheless, can be extremely complex. Some other important factors that will be considered when evaluating the use of molluscs as biomonitoring tools for the mining industry include:

1. Do molluscs occur naturally in mining impacted areas, and if not can they be artificially introduced in cages?

2. Are there differences in metal accumulation patterns between naturally occurring indigenous populations and transplanted molluscs?
3. Are there physiological constraints to using molluscs to monitor areas receiving mining discharge, e.g. ambient pH, Ca concentrations, etc.?
4. Are molluscs useful for detecting short-term pollution events (episodic events) or long-term trends in the receiving environment?
5. Can tissue metal concentrations be related to different metal sources (sediment or water) in the aquatic environment?

These questions will be addressed in subsequent sections and the answers will provide a framework within which to evaluate the usefulness of molluscs as biomonitoring tools for the mining industry in Canada.

3.3.3 Conceptual framework for molluscs as biomonitors for the mining industry

3.3.3.1 Molluscs as sentinels.

Monitoring bioavailable metals over the long-term: Historical surveillance

This approach mimics that used in the Mussel Watch (O'Connor, 1992). The goal is to utilize populations of long-lived unionids to monitor changes in bioavailable metal concentrations over time. This approach would be particularly useful in monitoring metal release in areas where mining activities have ceased and to determine if further clean-up is required. This type of monitoring would require the use of indigenous populations of molluscs that are likely to be present in sufficient numbers for the duration of the monitoring program. Transplanted molluscs could also be used in this case provided they are collected from the same source population for the duration of the monitoring program.

Monitoring bioavailable metals over the short-term:

To determine the spatial extent of metal contamination - Transplanted molluscs could be deployed at specific sites and their tissue levels compared to characterize the metal contamination throughout the impacted area and identify contamination "hot-spots".

To determine the effectiveness of remedial measures - The effectiveness of remedial measures such as "liming" or waste water treatment could be determined with transplanted or indigenous mollusc populations.

3.3.3.2 Molluscs as effects-monitors

Monitoring metal-induced effects:

- Growth measurements (shell length, soft-tissue weights and growth rates) could be used to estimate the potential metal-induced effects at the organismal level.
- Condition measurements could be used to determine the overall health of the metal-exposed population relative to a control population.

The above measurements would have to be accompanied by measurements of potential confounding environmental variables including temperature and food quality and quantity.

3.4 Characteristics of Molluscs relevant to biomonitoring

The following is a brief description of the important aspects of the diversity and distribution, ecology, physiology, reproduction and growth of molluscs that should be considered when applying these organisms as biomonitoring tools. The review will focus on freshwater bivalves and briefly discuss marine species and freshwater gastropods.

3.4.1 Taxonomy and diversity

Living members of the Phylum Mollusca are divided into seven classes: 3 major classes Cephalapoda, Gastropoda and Pelecypoda and 4 minor ones Monoplacophora, Polyplacophora, Aplacophora, Scaphopoda.

Phylum Mollusca		
Classes	Description	Aquatic Environment
Gastropoda	Snails and Slugs	Freshwater and Marine
Pelecypoda = Bivalvia	Mussels[a] and Clams	Freshwater and Marine
Cephalapoda	Octopods, Cuttlefish and Squid	Marine
Monoplacophora	Limpet-like shells, *Neopilina*	Marine, some fossil families
Polyplacophora	Chitons	Marine
Scaphopoda	Tusk Shells	Marine
Aplacophora	worm-like bodies with spicules	Marine

[a] the terms mussel and clam are often used interchangeably, but mussels usually refers to bivalves that do not possess an external siphon and clams refers to those that do.

Roughly 110,000 species of freshwater and marine molluscs have been described, of which over 50,000 species of molluscs belong to the marine and freshwater subclass Prosobranchia and another 20,000 belong to the primarily terrestrial subclass Pulmonata of Class Gastropoda (Thorp and Covich, 1991). In North America there are about 49 genera (350 species) of freshwater prosobranch snails and 29 genera (150 species) of pulmonate snails (Thorp and Covich, 1991). Some pulmonate snails have re-invaded the freshwater environment from the terrestrial environment, using a modified portion of the mantle cavity as a lung.

Although, the majority of bivalves are marine (Russell-Hunter, 1983), the North American fauna of freshwater bivalves is the richest in the world, with over 260 species of mussels and clams (Thorp and Covich, 1991). There are an additional 6 introduced species of bivalves. Most of the freshwater bivalve species belong to the Superfamily Unionacea (227 native species, 2 introduced) and the rest belong to the Family Sphaeriidae (33 native, 4 introduced species). Of the introduced bivalve species, the zebra mussel, *Dreissena polymorpha*, inhabiting some of the Great Lakes, has achieved the most notoriety.

Class Bivalvia	
Superfamily	Family
Unionacea	Unionidae
Corbiculacea	Sphaeriidae
Dreissenacea	*Dreissenia* spp.

The distributions of freshwater molluscs in Canada are related to glacial and postglacial history and to specific characteristics of the species themselves (Clarke, 1981). Based on the distribution of freshwater molluscs throughout Canada, there are several zoogeographic regions; Atlantic coastal, Lake Erie-Lake St. Clair, Great Lakes-St. Lawerence, Red River-Assiniboine, Western Prairie, Pacific coastal, Beringian Refugium, Subarctic and Arctic (Clarke, 1981). Many freshwater mussel species and all large prosobranch snails require continuous waterways for successful migration and as a result highly endemic species have developed (McMahon, 1991; Clarke, 1981). After periods of glacial cover, re-invasion by molluscs was restricted to the post-glacial stream confluence (Clarke, 1981). Alternatively, smaller molluscs (sphaeriid clams and small snails) are thought to have been transported to different geographical areas by ingestion and regurgitation by migratory birds or attached to their feathers or muddy feet (Clarke, 1981). Sphaeriid clams may also be transported distances attached to the legs of large insects (McMahon, 1991; Clarke, 1981). Transport between drainage systems mediated by human vectors (e.g. boats and bilge water release) has been partially responsible for the successful, disasterous invasion of the zebra mussel into the Great Lakes and the spread of *Corbicula fluminea* throughout North America. Within drainage systems, migration of these exotic species occurs when water currents transport the actively swimming veliger larvae of *Dreissena polymorpha* and juvenile *Corbicula fluminea* (McMahon, 1991).

The distribution of bivalves species in Canada's provinces is shown in Table 3.1. The most abundant of the unionid species are *Elliptio complanata* in eastern Canada, *Lampsilis radiata radiata* on the prairies, and *Anodonta kennerlyi* and *Margaritifera*

falcata in British Columbia (Clarke, 1981). Among the gastropods, the pulmonate *Stagnicola elodes*, Family Lymnaeidae, is the most common species in Canada (Clarke, 1981).

Endangered and Threatened Species

The conservation status of molluscs will play a significant role in their usefulness as biomonitoring tools. Species listed as threatened may have harvesting limits and/or moratoriums that prohibit their collection for any reason (scientific or otherwise). Evaluation of the status of mollusc diversity has only recently been considered in Canada with the formation of the Mollusca and Lepidoptera Subcommittee under the umbrella of the Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Unfortunately, no comprehensive assessments of the status of molluscs in Canada have been completed and only scattered information exists.

One of the most recent and comprehensive accounting of the conservation status of mollusc species in North America is provided by the American Fisheries Society (AFS). It provides a list of 297 native freshwater mussels that occur throughout North America, of which 213 taxa (71.7%) are considered endangered, threatened, or of special concern (see end of Table 3.1 for definitions) (Williams et al. 1993). Of the 52 native species found in Canada, 17 taxa (33%) are endangered, threatened or of special concern and another 5 species have undetermined status (Table 3.1). The remaining 30 mussel species are considered currently stable by the AFS. The difficulty with this assessment by the AFS is that the conservation status of each mussel species is based on its status across all of the provinces and states in which they occur. Therefore, the results are not specific to the status of mussels in Canada.

In the United States, over 25 unionid species have been put on the Federal Register's Endangered species list, with additional species on state lists and further species being added yearly (McMahon, 1991). Factors affecting their decline include habitat destruction from dams, channel modifications, siltation, and the introduction of non-indigenous species as well as overharvesting by the freshwater pearling industry (Williams et al. 1993; McMahon, 1991).

Table 3.1. Distribution and conservation status of freshwater mussel species in Canada and the provinces in which they occur. Adapted from Williams et al. (1993).

Mussel Species	Conservation Status	Provinces
Margaritifera falcata	undetermined	British Columbia
M. margaritifera	special concern	New Brunswick, Nova Scotia, New Foundland, PEI, Quebec
Actinonaias ligamentina	currently stable	Ontario
Alasmidonta heterodon	endangered	New Brunswick
A. marginata	special concern	Ontario
A. undulata	special concern	New Brunswick, Nova Scotia, Ontario, Quebec
A. varicosa	threatened	New Brunswick, Nova Scotia
A. viridis	special concern	Ontario
Amblema plicata plicata	currently stable	Manitoba, Saskatchewan, Ontario
Anodonta beringiana	undetermined	Yukon Territory, British Columbia
A. implicata	currently stable	New Brunswick, Nova Scotia, Quebec
A. kennerlyi	undetermined	Alberta, British Columbia
A. nuttaliana	undetermined	British Columbia
Anodontoides ferussacianus	currently stable	Manitoba, Ontario, Quebec, Saskatchewan
Cyclonaias tuberculata	special concern	Ontario
Elliptio complanata	currently stable	New Brunswick, Nova Scotia, Ontario, Quebec
E. dilatata	currently stable	Ontario
Elliptiodeus torulosa rangiana	endangered	Ontario
E. triquetra	threatened	Ontario
Fusconaia flava	currently stable	Manitoba, Ontario
Gonidea angulata	undetermined	British Columbia
Lampsilis cariosa	threatened	New Brunswick, Nova Scotia
L. fasciola	currently stable	Ontario
L. ovata	special concern	Manitoba, Ontario, Quebec, Saskatchewan
L. radiata radiata	currently stable	New Brunswick, Nova Scotia, Ontario, Quebec
L. siliquoidea	currently stable	Alberta, Manitoba, Northwest Territories, Ontario
L. complanata complanata	currently stable	Alberta, Manitoba, Ontario, Saskatchewan
L. compressa	currently stable	Manitoba, Ontario, Quebec, Saskatchewan
L. costata	currently stable	Manitoba, Ontario, Quebec
Leptodea fragilis	currently stable	Ontario, Quebec
L. ochracea	special concern	New Brunswick, Nova Scotia
Ligumia nasuta	special concern	Ontario
L. recta	special concern	Manitoba, Ontario, Quebec, Saskatchewan

Table 3.1. Con't.

Obliquaria reflexa	currently stable	Ontario
Obovaria olivaria	currently stable	Ontario, Quebec
O. subrotunda	special concern	Ontario
Pleurobema coccineum	currently stable	Ontario
Potamilus alatus	currently stable	Manitoba, Ontario, Quebec
Ptychobranchus fasciolaris	currently stable	Ontario
Pyganodon cataracta	currently stable	New Brunswick, Nova Scotia, Ontario, PEI, Quebec
P. fragilis	currently stable	New Brunswick, Nova Scotia, New Foundland,
P. grandis (formerly known as *Anodonta grandis grandis*)	currently stable	Quebec, Alberta, Manitoba, Northwest Territories, Ontario, Quebec, Saskatchewan, Yukon Territory
Quadrula pustulosa pustulosa	currently stable	Ontario
Q. quadrula	currently stable	Manitoba, Ontario
Simpsonaias ambigua	special concern	Ontario
Strophitus undulatus	currently stable	Manitoba, New Brunswick, Nova Scotia, Ontario, Quebec, Saskatchewan
Toxolasma parvus	currently stable	Ontario
Truncilla donaciformis	currently stable	Ontario
T. truncata	currently stable	Ontario
Utterbackia imbecillis	currently stable	Ontario
Villosa fabalis	special concern	Ontario
V. iris	currently stable	Ontario

Endangered - species or subspecies are in danger of extinction throughout a significant portion of its range.
Threatened - species or subspecies likely to become endangered throughout a significant portion of its range.
Special concern - species or subspecies may become endangered or threatened by relatively minor disturbances to its habitat and deserves careful monitoring.
Undetermined - species or subspecies whose historic and current distribution and abundance has not been evaluated in recent years.
Currently stable - species or subspecies whose distribution and abundance may be stable, or it may have declined in portions of its range but is not in need of immediate conservation.

The consequences of the declining status of mussel species in Canada for the use of molluscs as biomonitoring tools for the Canadian mining industry are significant, especially for long-term biomonitoring programs. The conservation status of individual

mollusc species in Canada must be determined prior to their inclusion in a national monitoring program.

3.4.2 Ecology

The utility of molluscs as biomonitoring tools depends in part on their ability to successfully inhabit areas influenced by mining activities. Although it is impossible to characterize the aquatic environment typical of all mining areas there are some similar traits including high metal concentrations, low pH due to smelting processes and acid mine drainage (AMD), and high turbidity and silt loads from the under-water disposal of waste rock and till. The range of habitats occupied by molluscs are described in the following section.

3.4.2.1 General. Freshwater molluscs have a relatively ubiquitous distribution inhabiting a wide range of aquatic environments, from small ponds to large lakes and rivers. They are usually absent from extremely cold alpine lakes and streams, acidic waters, or grossly polluted areas (Elder and Collins, 1991; Clarke, 1981). Mollusc distribution can be limited by very low concentrations of dissolved salts including Ca, needed for shell production (Elder and Collins, 1991). They are restricted to more alkaline waters and are rarely found in waters more acidic than pH 6. Molluscs generally have high oxygen demands that tends to exclude them from anoxic or near-anoxic conditions (Elder and Collins, 1991). Nevertheless, some species of freshwater bivalves have adapted to hypoxic or even anoxia conditions (McMahon, 1991). This allows the bivalves to occur below the thermocline of stratified lakes or above reducing substrata with heavy organic loads or in areas with large numbers of molluscs (McMahon, 1991).

Fish are the primary predators of molluscs, although only a few species, such as suckers, perch and whitefish, rely on them for a significant portion of their diet (Elder and Collins, 1991). Snails are consumed by ducks, shore birds, and occasionally amphibians. Both mussels and clams are consumed by terrestrial species, such as muskrats and turtles (Elder and Collins, 1991).

Marine gastropods can be found living between tide marks on the seashore, on the ocean bottom at all depths, and drifting in the plankton near the sea surface (Russell-Hunter, 1983). The gastropod *Littorina* live high on the seashore in zones wetted by sea spray (Russell-Hunter, 1983). Most of the primitive marine bivalves are infaunal, burrowing into soft substrates, but several superfamilies are epifaunal, mostly attached to rocks (Russell-Hunter, 1983).

3.4.2.2 Bivalves. The distribution of freshwater bivalves is determined to some extent by sediment type. Unionids are most often found in stable, coarse sand or sand-gravel mixtures and are usually absent from silted areas (McMahon, 1991). Mussel fauna in areas receiving silt loads and acid discharges from mines have been adversely affected or totally extirpated (McMahon, 1991). Areas where water velocities are low enough to allow for sediment stability and high enough to prevent excessive siltation tend to have successful unionid populations (McMahon, 1991). Sphaeriids, on the other hand prefer substrates with fine particles, although there are some species differences in the optimal particle sizes (McMahon, 1991). A few unionids, *Pyganodon grandis*, *Lampsilis anodontes*, and *L. radiata*, are found in sand-mud substrates (McMahon, 1991).

Water depth is a factor in the distribution of freshwater bivalve species. Most unionaceans, as well as *Sphaerium* and *Musculim* sphaeriid species prefer water depths less than 10 m, but can be found deeper if oxygen concentrations are sufficient (McMahon, 1991). Two unionid species, *Elliptio complanata* and *Pyganodon grandis*, that inhabit lakes in northern latitudes, are tolerant of low oxygen levels resulting from long periods of ice-cover (McMahon, 1991). Due to their ability to regulate oxygen uptake, many species of the sphaeriid *Pisidium* can be found below the epilimnion of lakes in poorly oxygenated areas. Profundal sphaeriids such as *Sphaerium simile* and *Pisidium casertanum* are somewhat oxygen independent; however, long periods of hypoxia and anoxia have deleterious effects on their growth and reproduction (McMahon, 1991).

Unionaceans grow and reproduce over a pH range of 5.6 - 8.3, with a pH of 4.7-5.0 being the absolute lower limit (McMahon, 1991). Sphaeriid species are somewhat

more tolerant of low alkalinity habitats. A pH of 5.0 has been identified to produce maximal growth and reproduction in the sphaeriid *Musculium partumeium* (McMahon, 1991). The pH ranges that limit bivalve success are linked to the availability of Ca. The minimal Ca concentrations tolerated by freshwater bivalves are 2-2.5 mg L^{-1}, with sphaeriid species occurring at the lower concentration ranges (McMahon, 1991).

Bivalve species distributions are limited by temperature. If water temperatures are too high, molluscs may perish or have their reproductive cycles disrupted, but if water temperatures are only slightly elevated over normal, reproduction may actually increase (Clarke, 1981). *Corbicula fluminea* is not found in drainage systems that experience water temperatures less than 2°C (McMahon, 1991). *Dreissena polymorpha* has maximal temperature limits of 25-27°C above which larval development is prevented (McMahon, 1991). No studies describing specific temperature limits for other unionid species could be found.

3.4.3 Basic morphology, physiology, reproduction and growth

Life-history characteristics are used to evaluate the different mollusc groups (i.e. unionids, sphaeriids and *Dreissena polymorpha*) on their practical application as biomonitors of spatial and temporal change. Factors such as maturation age, life span, and biomass turnover rates are considered. In addition, aspects of molluscan morphology, physiology, reproduction, and growth are reviewed in light of the important role they play in determining metal uptake, accumulation and metabolism.

3.4.3.1 Bivalves. Unlike snails, bivalves have limited mobility and their body is encased in two hinged, calcareous shell valves. They possess enlarged gills with elongated, ciliated filaments for filter feeding (McMahon, 1991). Bivalves are laterally compressed and dorso-ventrally expanded, which combined with their spade-like foot make them highly efficient at burrowing into the substrate. North American freshwater bivalves generally have short siphons and as a result are normally found buried to depths where the posterior shell margin is either slightly below or above the sediment surface (McMahon, 1991). The zebra mussel *Dreissena polymorpha* does not burrow into the

sediment, but instead uses byssus threads made of proteinaceous materials to anchor itself to many different types of surfaces.

Life-history traits and reproduction

A summary of important life-history traits of freshwater bivalves is shown in Table 3.2. Almost all North American freshwater bivalves are ovoviviparous, brooding embryos through early development stages in the gill. *Dreissena polymorpha* is the only exception, releasing both sperm and eggs externally, leading to a free-swimming veliger larval stage (McMahon, 1991). The majority of unionids are gonochoristic and all sphaeriids are hermaphroditic (McMahon, 1991). Self-fertilization does occur, but most fertilization results when sperm released into the water is taken up in the inhalent currents of surrounding individuals and transported to the unfertilized eggs retained in the gills. Temperature is the main stimulus for initiating reproduction. Unionaceans release a large number of parasitic glochidium larvae once per year that require attachment to a fish host prior to metamorphosing into a juvenile (McMahon, 1991). Fish hosts of glochidia are species specific, and some unionid species can have more than one fish host species (Kat, 1984). If the glochidium is not compatible with the fish species, an immune reaction in the fish causes the glochidium to be sloughed off (McMahon, 1991). Because of this specificity, the reproductive success of certain unionid species depends on the success of their fish host.

Age-specific survivorship among juvenile unionids tends to be low, improving with age (Table 3.2). Survivorship in adult *Anodonta anatina* (5-7 year olds) was 81-86% (McMahon, 1991). In unionids, the shell growth rate declines exponentially with age, but the rate of tissue biomass accumulation remains constant or actually increases with age (McMahon, 1991). Natural unionid populations tend to be dominated by large adults, due to their high adult survivorship, long life spans and low juvenile survivorship. Biomass turnover times in these types of populations are extremely long ranging from 1790-2850 days (McMahon, 1991). In contrast, turnover times for sphaeriids (27-1970 days) and *Dreissena polymorpha* (53-870 days) are much shorter. These life-history traits make unionids highly susceptible to human perturbations (McMahon, 1991).

Table 3.2. Summary of life-history charcteristics of North American freshwater bivalves, Unionacea, Sphaeriidae, and *Dreissena polymorpha*. Adapted from McMahon (1991).

Life History Trait	Unionacea	Sphaeriidae	*Dreissena*
Life span	<6 - >100 yr (species dependent)	<1 - >5 yr (species dependent)	4 - 7 yr
Age at maturity	6 - 12 yr	> 0.17 - <1 yr (1 yr in some species)	1 - 2 yr
Reproductive mode	gonochoristic (a few hermaphroditic species)	hermaphroditic	gonochoristic
Growth rate	rapid prior to maturity, slower thereafter	slow relative to unionids	rapid throughout life
Fecundity (young/average adult/breeding season)	200,000 - 17,000,000	3 - 24 (*Sphaerium*) 2 - 136 (*Musculim*) 3 - 7 (*Pisidium*)	30,000 - 40,000/female
Juvenile size at release	very small 50 - 400 µm	large 600 - 4150 µm	extremely small 40 - 70 µm
Relative juvenile survivorship	extremely low	high	extremely low
Relative adult survivorship	high	intermediate	intermediate 26 - 88 %/yr
No. of reproductive efforts/yr	1	1 -3 (continuous in some species)	1 (2 - 8 months long)
Assimilated energy respired	-	21 - 91 % (avg. = 45 %)	-
Nonrespired energy in growth	85.2 - 97.5 %	65 - 96 % (avg. = 81 %)	96.1 %
Nonrespired energy in reproduction	2.8 - 14.8 %	4 - 35 % (avg. = 19 %)	4.9 %
Turnover time in days (mean standing crop biomass: biomass production/day ratio)	1790 - 2849	27 - 1972 (generally < 80)	53 - 869 (depends on habitat)

Sphaeriid species show a greater degree of variability in life history traits among species compared to the unionids. Sphaeriids are characterized by early maturation, rapid growth, and reduced reproductive effort, which equips them to deal with the environmental stresses of their habitat (McMahon, 1991). Fertilized embryos develop in brood chambers in the gills and are supplied with maternal nutrients allowing for considerable growth and development prior to release (McMahon, 1991). In contrast to the unionids, sphaeriids generally produce a small number (3-24 young/adult *Sphaerium sp.*) of large offspring (0.6-4.15 mm) (McMahon, 1991). The production of fewer young normally suggests that a species has adapted to relatively stable habitats. However,

sphaeriids are most often found in shallow ponds and profundal portions of lakes which are exposed to extended periods of hypoxia and drying.

Balancing the amount of energy spent on shell production (up to one-third of total energy related to growth) and that allocated to tissue growth is an adaptive strategy developed by bivalves. Fast-growing, thin-shelled species (most sphaeriids) devote less energy to growth and reproduction compared to slower-growing, thick-shelled species (most unionids) (McMahon, 1991).

Nutrition and filtration rates

Of the three distinct subclasses that make up the Class Bivalvia the most common are the filter-feeding lamellibranchs and deposit-feeding protobranchs. Most freshwater bivalves are suspension feeders, filtering unicellular algae, bacteria and suspended detrital particles from the pallial water flow across the gill. The material filtered out by the gill is passed to the labial palps for cilia-mediated sorting of food from non-food before being carried on ciliary tracts to the mouth. The stomach and style have pH levels ranging from 6.0-6.9, style acidity varying with phase of digestion.

Filtration studies on both marine and freshwater bivalves report that most species can retain particles greater than 4 μm in diameter with nearly 100% efficiency (Table 3.3) (Tankersley and Dimock, 1993). The zebra mussel could retain particles as small as 0.7 μm in diameter, although the preferred size range was considerably larger (5 - 35 μm) (Sprung and Rose, 1988). Particle ingestion appears to be affected by food type with some bivalves selecting certain algal species (McMahon, 1991). Filtration rates are affected by particle size and particle concentration (Tankersley and Dimock, 1993). For example, an increase in filtration rate was observed in the freshwater mussel, *Pyganodon cataracta*, as the size of particles they were being fed was increased over a range from 2 μm to over 9 μm in diameter (Tankersley and Dimock, 1993). Tankersley and Dimock (1993) also reported differences in filtration rate depending on reproductive status, whereby females had significantly higher filtration rates during pre-brooding periods than during brooding.

Table 3.3. Bivalve filtration rates and size of particles most efficiently retained. Range of particles retained are shown in parentheses.

Species	Particle Size μm	Filtration Rate	Reference
Mytilus edulis		119 L g^{-1} d^{-1}	Widdows et al. 1995
Pyganodon cataracta	8	4 L g^{-1} d^{-1}	Tankersley and Dimock, 1993
Dreissena polymorpha	5 (5 - 35)	5.5 L d^{-1}	Sprung and Rose, 1988
Dreissena polymorpha	>1		Jorgensen et al. 1984
Unio pictorum	5-8		Jorgensen et al. 1984
Anodonta cygnea	6-8		Jorgensen et al. 1984
Elliptio complanata	5		Paterson, 1984
Elliptio complanata	(8 - 80)		Tessier et al. 1984

Metabolism

Metabolic rates in bivalves correspond to seasonal changes in temperature, where the highest rates occur in the summer and the lowest in the winter months (McMahon, 1991). Sphaeriids experience variable metabolic rates corresponding to growth and reproductive cycles while unionids do not. Unionids do not provide embryos with maternal nourishment, although the physical presence of the developing glochidia in their gills has been found to reduce filtration rates and particle-retention efficiencies (Tankersley and Dimock, 1993). There is some evidence that bivalves have diurnal activity patterns where the most active periods occur at night, possibly related to feeding and vertical migration cycles of zooplankton that could conceivably increase epilimnetic particle concentrations at night (McMahon, 1991). Distinct annual biochemical cycles have been found in freshwater bivalves and are thought to be related to reproduction (McMahon, 1991). Maximal levels of whole body protein, glycogen and lipid are reached during gametogenesis and gonad development and then drop to a minimum after glochidial release.

3.5 Relationship between concentrations of metals in sediment and water and concentrations in molluscs

It is important that a biomonitor strongly accumulate the contaminant of interest and that the tissue contaminant concentrations be directly proportional to average ambient bioavailable contaminant concentrations. For many organic contaminants, relatively simple correlations have been determined (e.g. fugacity/hydrophobicity constants) that hold over a range of environmental conditions (Clark et al. 1990). However, the development of "simple" correlations for metals has been more difficult. Metal bioavailability and metal accumulation by molluscs change in response to environmental conditions and from one species to another. Interpreting tissue metal concentrations in the aquatic environment requires an understanding of the factors that influence metal availability and determine metal accumulation in molluscs (Fig. 3.1).

The availability of a metal is determined by its speciation which may be very sensitive to changing environmental conditions (e.g. pH, presence of natural inorganic and organic ligands). Reactions affecting metal speciation may occur within organisms, on their surface, in solution, and on the surface of particles, making understanding metal behavior difficult. The availability of metals to molluscs will be a function of both the source of metal exposure (dissolved or particulate phase) and the geochemical processes that control metal availability within each phase.

Metal accumulation in molluscs is also affected by individual biological characteristics and aspects of mollusc physiology. Individual biological characteristics that determine organism response to metal exposure typically include aspects of growth and reproduction, as well as behavior. Biological characteristics are of particular importance when comparing metal accumulation in indigenous populations to accumulation in molluscs that have been transplanted and/or caged. Other factors affecting accumulation that are a function of mollusc physiology include the route of metal uptake, rates of uptake, assimilation/retention efficiencies, and detoxification and elimination processes.

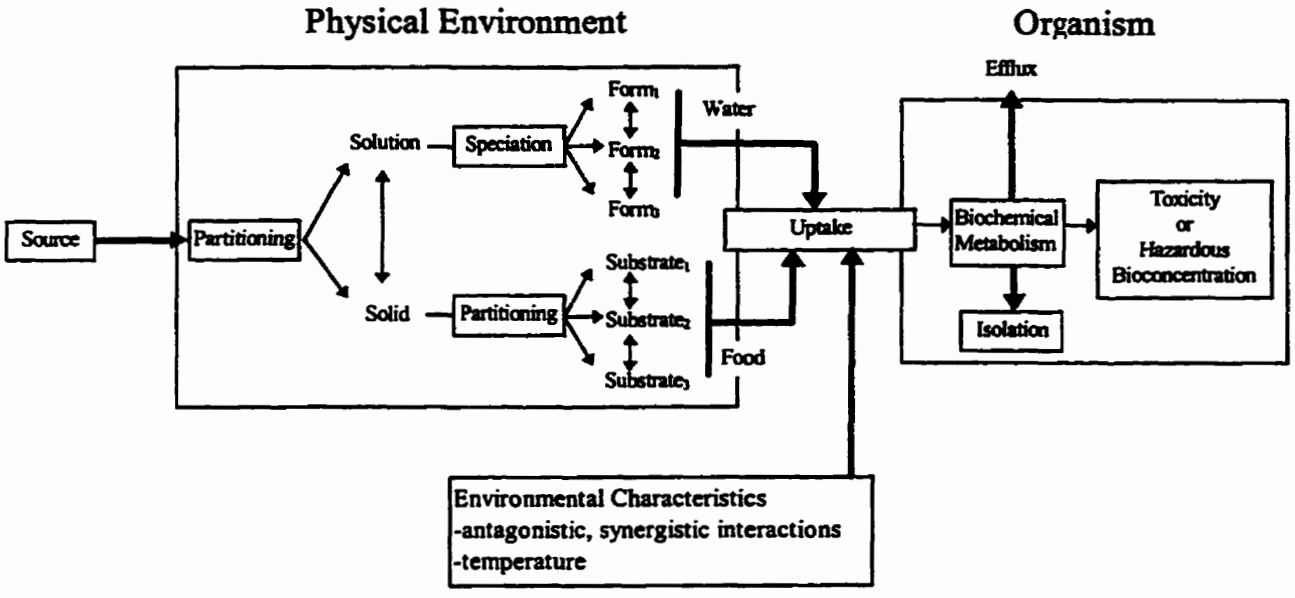


Figure 3.1. Processes affecting metal availability and accumulation in aquatic organisms. Redrawn from Luoma (1983).

3.5.1 Bioaccumulation

The accumulation of metals by molluscs can be divided into three phases: 1) metal uptake, 2) metal transport, distribution, and sequestration within the body, and 3) metal excretion (may or may not occur) (Rainbow and Dallinger, 1993). The role that a metal plays in mollusc physiology (essential vs. non-essential) and the ability of the different mollusc species to regulate metals influence the accumulation processes (Rainbow and Dallinger, 1993). Molluscs able to regulate metals show no significant change in body metal content over time on exposure to elevated concentrations of bioavailable metal. This may be achieved by excluding all metal (no absolute uptake), a strategy possible for the short-term but not long-term, or by altering the rate of metal excretion to match the rate of metal uptake (Rainbow and Dallinger, 1993). Unlike many other invertebrates, molluscs have limited ability to reduce uptake by reducing permeability of the outer surfaces. Filter-feeding bivalves have huge gill surfaces which are bathed in the external medium to facilitate food uptake and respiration. The molluscs' inability to strongly regulate metals is an important characteristic that many argue makes them good biomonitors.

The following is an overview of metal accumulation in molluscs including uptake (kinetics), elimination (assimilation/retention efficiencies) and storage (distribution among tissues, organs and cytosol).

3.5.1.1 Uptake. The rate of metal uptake has consequences for the use of molluscs as biomonitoring tools. For example, molluscs with rapid uptake rates might be suited to monitoring short-term episodic events (e.g. spills) compared to molluscs with considerably slower uptake rates that would be more suited to monitoring long-term fluctuations in bioavailable metal concentrations. Metal uptake rates are influenced by characteristics of the mollusc species (life strategies, age, growth rate, reproductive status) as well as a series of factors related to the metal species (i.e. route of uptake, partition coefficient (K_d)). Steady state is obtained when uptake rates equal excretion rates.

Metal uptake in bivalves may occur through the transport of dissolved metals across the gill or by ingestion of metal-laden particles filtered out by the gills. Consequently, uptake rates in bivalves are predominantly a function of filtering activity and absorption efficiency from the dissolved phase. Uptake rates vary from one mollusc species to the next and are metal specific. Laboratory studies with *Mytilus edulis* found uptake rates for the different metals in the order Ag>Zn>Am ≈ Cd>Co (Wang et al. 1996).

The life strategy of the freshwater zebra mussel *Dreissena polymorpha* (small, high biomass turn-over rate, high growth rates) makes it more suitable to monitoring short-term fluctuations in metal concentrations than other, older, long-lived unionids. Indigenous and caged zebra mussels, used to monitor metal concentrations in a Cu-contaminated reservoir in France, showed marked fluctuations in metal concentrations in their tissues (including decreases) reflecting changes in water contamination on a monthly basis (Mersch et al. 1996).

Larger, slower growing unionids tend to respond more slowly to changes in metal concentrations. Uptake rates calculated for *Pygandon grandis* transplanted from uncontaminated Lake Brompton to Cd- and Cu-contaminated Lake Joannes in Quebec for 130 days, were 0.11 μg Cd $g^{-1}d^{-1}$ and 0.18 μg Cu $g^{-1}d^{-1}$, assuming a linear increase in Cd

uptake (Tessier et al. 1987). Based on these rates of uptake it would take 1 (Cu) and 2 (Cd) years for metal concentrations in the transplanted Lake Brompton mussels to reach the levels found in indigenous Lake Joannes mussels. Tessier et al. (1993) reported that freshwater bivalves (*Pyganodon grandis*) transplanted along a contamination gradient (Cd, Cu and Zn) had not reached steady state with their new environment after 1 year. Similar results were found by Couillard et al. (1995a), except that tissue Zn concentrations in transplanted *Pyganodon grandis* rapidly reached those concentrations found in the indigenous population. This may have been due to the essential role of this metal in mollusc physiology. Cadmium concentrations in indigenous *Pyganodon grandis* in Lake 382 in the Experimental Lakes Area (ELA), Canada, receiving epiliminetic additions of Cd (average ambient water [Cd] 1987 ~85 ng L^{-1}), continued to increase 120 days after metal additions had begun (Malley et al. 1989). In another experiment, Cd concentrations in *Pyganodon grandis* transplanted from pristine Lake 377 in the ELA and caged on the sediment in Lake 382, were still increasing after 26 days (average ambient water [Cd] 1988 ~100 ng L^{-1}) (A.R. Stewart, unpublished data).

Uptake rates in marine species in the field appear to be much faster than those for freshwater species. Regoli and Orlando (1994) found that the marine mussel *Mytilus galloprovincialis*, transplanted to a metal contaminated site, accumulated Pb, Fe, and Mn (linear increase) and reached steady state metal concentrations in tissues after 14 days exposure.

Several laboratory studies on the kinetics of metal uptake in freshwater molluscs indicate that uptake rates and times to reach steady-state are much more rapid in the laboratory than in the field. However, these studies utilized unrealistically high water metal concentrations that may have resulted in uptake by different routes with different uptake kinetics than those expected for lower, more realistic metal concentrations in the natural environment (Hemelraad et al. 1986, 5 to 25 µg Cd L^{-1}; Tessier et al. 1994, 10 and 50 µg Cd L^{-1}; Jenner et al. 1991, ~50 µg Cd L^{-1}; Graney et al. 1984, 50 µg Cd L^{-1}).

3.5.1.2 Assimilation/Retention efficiencies. The net accumulation of a metal by molluscs is a function of its assimilation and retention efficiencies from food and water, respectively. Assimilation efficiency (AE) refers to the amount of metal retained from

particles relative to total amount ingested. Retention efficiency (RE) is the amount of metal adsorbed from the dissolved phase relative to the amount filtered. Estimates of AEs and REs for different mollusc species are critical for interpreting bioaccumulation studies, but are rarely calculated for freshwater species (Wang et al. 1996). These measurements provide a means to identify important sources of metals for bivalves under natural conditions and help explain tissue metal burdens. Recent work by Wang et al. (1996) and Fisher et al. (1996) using the marine mussels *Mytilus edulis* and *M. galloprovincialis* provides a comprehensive overview of factors affecting assimilation and retention efficiencies and provides comparisons between the laboratory and field. Some of the results are provided below and concentration ranges used in the experiments are shown in Table 3.4.

Retention efficiencies determined for laboratory-exposed *M. galloprovincialis* were generally higher than AEs for Ag, Am, Cd, inorganic Co, organic Co, Pb, and Zn (Fisher et al. 1996). Also, variability in REs was generally higher than for AEs for all the metals tested. These results suggest that the accumulation of the tested metals from the dissolved phase is greater than from the ingested solids, and that uptake from solution is more sensitive to changes in water chemistry.

Mytilus edulis fed radioisotope-tagged seston showed differences in Ag and Cd assimilation that were found to vary with the nutritional quality of ingested food particles (Wang et al. 1996). Silver was more efficiently assimilated and Cd was less efficiently assimilated from seston with higher algal content. Cadmium has been found to be assimilated with lower efficiency from green algae than from other algal species, possibly due to the rigid cell wall of the algae and resistance to digestion (Atkinson et al. 1972). Zinc, Am and Co were not appreciably affected by the quality of seston in the experiment. The AEs of ingested metals from natural seston were Ag (5 to 18%), Am (0.6 to 1%), Cd (8 to 20%), Co (12 to 16%) and Zn (32 to 41%) (Wang et al. 1996).

Assimilation and retention efficiencies from the laboratory and field were not appreciably different, except for Ag and Co (Fisher et al. 1996). For Ag, the AEs of mussels held in the laboratory were 5 times greater that those in the field. Silver binds strongly to sulfur in ligands in protein and so different environmental conditions (e.g. temperature) could affect protein metabolism, significantly affecting its absorption in

Table 3.4. Concentration ranges used in studies on assimilation and retention efficiency.

Fisher et al. 1996	Concentration ranges of dissolved trace elements[a]
Co	0.8 - 1 ng L^{-1}
Zn	3 - 6 ng L^{-1}
Cd	0.6 - 0.7 ng L^{-1}
Ag	860 ng L^{-1}
Pb	3 - 4 ng L^{-1}
Wang et al. 1996	**Concentration ranges of dissolved trace elements**
Co	0.1 - 10 µg L^{-1}
Zn	0.5 - 300 µg L^{-1}
Cd	0.1 - 10 µg L^{-1}
Ag	0.2 - 100 µg L^{-1}

[a]Values = Concentrations of added metals + background metal concentrations in 0.2 µm filtered Mediterranean seawater (Personal communication, N. Fisher, Stony Brook, New York).

mussels. Only Ag absorption in *Mytilus edulis* was found to be inversely related to temperature (5 vs. 15°C) (Fisher et al. 1996). This finding suggests that most laboratory determined assimilation efficiencies (AEs) could be used to estimate field situations. Further, efflux rate constants for metals in marine mussels derived from laboratory experiments were consistent with values obtained from field studies (Fisher et al. 1996).

3.5.1.3 Elimination. Metals taken up into molluscs are not necessarily incorporated into tissues or utilized in metabolism. Some metals are released immediately as feces and others are detoxified in the digestive gland and excreted. In the absence of elevated metal concentrations in the environment, metals may be progressively lost from the soft tissues and shell. The elimination of metals in molluscs has consequences for measuring impacts of improvements to waste-water treatment as well as trends in recovery of metal

contaminated systems, i.e., will tissue burdens decrease to reflect lower ambient metal concentrations?

Elimination rates were very slow in the freshwater bivalve *Pyganodon grandis* transplanted from contaminated Lake Joannes to relatively uncontaminated Lake Brompton. The half-life ($t_{\frac{1}{2}}$), i.e., or time to reduce the metal concentration at time zero by half, for Cu and Cd in *P. grandis* calculated over the 130 day transplant period was 315 and 143 days, respectively (Tessier et al. 1987). Cadmium elimination rates for several freshwater and marine clams and mussels are shown in Table 3.5. It appears that elimination rates vary among metals, mussel species, and tissues. A very general conclusion from these data is that if losses occur at all they do so slowly, with half-lives of 100 to 300 days. Half-lives for some marine species (*Mytilus californianus*) are considerably shorter than 100 days.

In a study that compared the elimination in the marine mussel *Mytilus galloprovincialis* caged in the field and in the laboratory, radioactive metal release (Ag, Am, Cd, inorganic Co, organic Co, Pb and Zn) generally followed a two-compartment exponential model (Fisher et al. 1996). Uptake from food (cultured marine phytoplankton) resulted in a rapid release of isotope, primarily in the form of fecal pellets. Alternatively, metals accumulated from water were lost more slowly as metal desorbed from the shell. A discrepancy in the efflux rates for whole mussels and for mussel shells was evident between laboratory and field mussels which was thought to be linked to epiphytic growth on the mussel shells in the field, thus limiting desorption (Fisher et al. 1996). For this reason, the authors suggested that efflux rate constants for soft tissues may be more relevant than those for shells. The authors also noted seasonal changes in efflux rates in mussels which they suggested might be linked to seasonal variations in food availability (Fisher et al. 1996).

Neither the route of uptake (water vs. natural seston) nor exposure time (12 hrs. vs. 6 days) had a significant effect on the efflux rates in *Mytilus edulis* exposed to radioisotopes Ag, Am, Cd, Co, Se, and Zn, suggesting that assimilated radiotracers were able to partition rapidly into even the least labile compartment (Wang et al. 1996). Conversely, Fisher et al. (1996) found that efflux rate constants for radiolabeled Co and Am in *Mytilus galloprovincialis* soft parts were 2-3 times greater following uptake from

Table 3.5. Elimination of metals from the soft tissues of bivalves.

Species	Metal	Experiment	Metal loss pattern	Reference
Pyganodon grandis	Cu	field	$t_{½}$= 143 d	Tessier et al. 1987
Pyganodon grandis	Cd	field	$t_{½}$= 315 d	Tessier et al. 1987
Dreissena polymorpha	Cd	laboratory	0% loss, 50 d depuration	Bias and Karbe, 1985
Unio pictorium	Cd	laboratory		Jenner et al. 1991
kidney			0 % loss, 203 d	
gill			$t_{½}$= 203 d	
hepatopancreas			30 % loss (exponential) , 203 d	
Mytilus californianus	^{65}Zn	field	$t_{½}$= 76 d	Young and Folsom, 1967
Mytilus californianus	^{65}Zn	field	$t_{½}$= 380 d	Seymor and Nelson, 1973
Mytilus edulis	^{65}Zn	field	$t_{½}$= 277 d	Seymor and Nelson, 1973
Mytilus edulis	Cd	laboratory	$t_{½}$= 14-29 d	Scholz, 1980
Mytilus edulis	Cd	laboratory	$t_{½}$= 96-190 d	Borchardt, 1985
Crassostrea virginica	^{65}Zn	field	$t_{½}$= 347 d	Wolfe, 1970
Mercenaria sp.	Cd	laboratory	0% loss, 64 d depuration	Robinson and Ryan, 1986

food than from water, although there were no differences found for the other metals, Zn, Cd, Ag, and Pb.

3.5.1.4 Storage. The processes that control the distribution of metals within tissues and cells affect both bioaccumulation itself and any adverse impact resulting from bioaccumulation. Thus, the understanding of metals storage is critical to interpreting possible risk posed by accumulated metals. The distribution of metals among tissues and within cells continuously changes following metal exposure. The route of uptake (solution vs. particulate), the mechanism of transport across the cell membrane (facilitated or diffusive transport; passive or active-transport), the affinity of the metals for intracellular ligands (e.g. metallothionein), and, most importantly, the role of the metals (essential vs. non-essential) determine where the metals are sequestered and how they metabolized.

The distribution of Cd concentrations in tissues of naturally occurring unionids has been reported to follow the sequence: kidney>digestive gland>gills>> mantle=mantle edge=labial palps>guts/gonads complex>foot and adductor muscle (Hemelraad et al. 1986; Salánki et al. 1982; Manly and George, 1977). The kidney and midgut gland contain approximately half of the total body burden for Cd. This distribution can dramatically change after exposure. Concentrations of Cu, Zn and Pb in *Elliptio complanata* collected from lakes in the Rouyn-Noranda mining region of Quebec were highest in the gills and mantle, intermediate in the hepatopancreas (digestive gland) and lowest in the foot and adductor muscle (Tessier et al. 1984). The gills (for Cu, Zn, Mn) and mantle (for Fe) made the highest contribution to the metal body burden (Tessier et al. 1984). The highest proportion of the total Cd body burden was also found in the gill (40%) of another bivalve species, *Pyganodon grandis* collected from lakes in the Rouyn-Noranda mining region (Tessier et al. 1993). A different distribution of Cd was found in indigenous *Pyganodon grandis* exposed *in situ* to ^{109}Cd (~83 ng L^{-1}) over the ice-free season (~114 d), where the order of concentrations within the tissues followed the sequence: kidney> remains of soft tissues[1]>gill>foot>mantle (Malley et al. 1989).

[1] Remains of soft tissues include gonads, gut, adductor muscles and digestive gland.

However, the order of Cd in tissues expressed as a proportion of the total body burden followed the sequence: remains of soft tissues>gill>mantle, kidney>foot, which is similar to that found in other indigenous bivalves exposed to elevated metals.

Fisher et al. (1996) found that the distribution of metals within *Mytilus galloprovincialis* was affected by the source of metal exposure (dissolved vs. particulate) in the laboratory. A greater fraction of the metal obtained from the dissolved phase was associated with the shell, while a greater fraction of that obtained from ingested food was found in soft parts such as the digestive gland. Within soft-tissues, metal obtained from the dissolved phase was predominantly in the gills at the end of the metal exposure and beginning of the depuration period, whereas metals obtained from food were most found in the digestive gland. There were no significant differences in the distribution of radioisotope from either source in the mantle, gills, or adductor muscle.

The subcellular distribution of metals in molluscs depends on the metal and the duration and degree of exposure. Couillard et al. (1995b) report the subcellular (cytosolic) distribution of Cd and Cu in the gills of *Pyganodon grandis* transplanted along a contamination gradient in the Rouyn-Noranda Region of Quebec. After 14 days exposure, Cd was associated primarily with the high molecular weight (HMW, >15 kDa) ligands in the gills. After 90 days, all of the Cd had then shifted to the moderate molecular size fraction (15 - 3 kDa, Metallothionein fraction (MT)). After 400 days, a considerable portion (74%) of the Cd was found in the low molecular weight (LMW) fraction. In contrast, cytosolic Cu, measured only after 90 days exposure, was found primarily bound to the HMW pool (85%). Based on experiments with *Crassostrea virginica*, Roesijadi and Klerks (1989) suggested that Cd binding in the gill is controlled by competition among available ligands displaying varying affinities for the metal, MT having the highest. After a lag period during which the MT is synthesized, the majority of Cd is found associated with the MT fraction. The observed shift from the MT fraction toward the LMW after 400 days exposure may have been an example of "spillover" of excess metals from the MT pool. Couillard et al. (1995b) and Couillard (1996) discuss the toxicological significance of the "spillover" effect.

Cytosolic shifts in the distribution of Ag, Cu and Zn were also found in whole bivalves, *Macoma balthica*, collected monthly from January 1981 to June 1982 in South

organic pollution literature but have been used less to describe metal pollution. The Canadian Toxic Substances Management Policy uses BCFs to determine the hazard posed by substances that are bioaccumulative and the associated action that should be taken by the government given any particular BCF (trigger levels). Recently, the use of BCFs in assessing metal contamination has been reviewed and given an unfavorable recommendation. Chapman et al. (1996) suggest a number of reasons why BCFs are not appropriate for regulating metal contamination: 1) some metals (e.g. Co, Cu, Fe, Mn, Mo, Zn) are essential for health and organisms regulate these metals over a range of environmental metal concentrations; therefore, BCFs will vary according to the external metal concentration; 2) essential metals have a double "toxicity" threshold (due to shortages and excesses); organisms will concentrate essential metals when external concentrations are low resulting in high BCFs, and; 3) BCFs assume that tissue metals concentrations are at steady-state, a condition that is difficult to determine in the field. Similar limitations exist for corresponding Bioaccumulation Factors (BAFs) which are calculated for steady-state environmental concentrations (metal source is not specified - could be sediment, water or food).

3.5.1.6 Summary

1. Metal uptake rates are faster in zebra mussels and sphaeriid species than in unionids. Time for tissue metal concentrations in unionids to reach steady-state ranges from 2 weeks to over 1 year.
2. Laboratory and field studies indicate that *Mytilus* species accumulate metals more efficiently from the dissolved phase than from food and that accumulation of Ag and Cd from food is dependent on the nutritional quality of the food.
3. Elimination rates vary according to the metal, species, target organ and season. A general estimate for a 50% reduction in tissue concentration is 100 to 300 days.
4. Metal storage varies for different metals, route of uptake (dissolved vs. food), and over the exposure period. Metals from the water or food are generally first accumulated in the gill and digestive gland, respectively, and then ultimately stored in the kidney. Subcellular metal distributions are metal specific and tend to change in response to cytosolic metal concentrations.

5. Metal BCFs or BAFs can be potentially misleading and should not be used to describe the relationship between tissue-metal concentrations and environmental exposures.

3.5.2 Bioavailability

Unlike the case for organic pollutants, relationships between aquatic organisms and environmental concentrations of metals are not easily defined by a few characteristics of the external medium and organism (i.e. organic content of sediments, lipid content of organisms). Bioaccumulation of metals by aquatic organisms cannot be easily related to total pollutant, nor is it possible to measure one chemical fraction that is universally and exclusively the bioavailable fraction for any metal (Luoma, 1996). There is no universally accepted approach to explain how metals in sediments become bioavailable to aquatic organisms (Luoma, 1996). A number of different theories and models have been developed and help to explain the bioavailability of different metal groups, e.g. free-ion activity correlates with Cu, Cd, Pb or Zn bioavailability; chloro-complexes of dissolved Ag may be able to bypass specific transport pathways and passively diffuse across lipid membranes; methylated forms of Hg and Sn demonstrate enhanced bioavailability via specific organo-complex transport and sequestration (Luoma, 1996). Depending on the metal in question, different theories must be considered. Campbell and Tessier (1996) provide an excellent overview of the factors controlling metal bioavailability in freshwater systems that will be discussed in the following section.

Metals in contaminated sediments are thought to be taken up by aquatic life in two ways: indirectly (i.e. by partitioning of the metals into the ambient water, followed by their assimilation from the aqueous phase), and directly (e.g. by digestion of the sediments and assimilation of the metals from the gut) (Campbell and Tessier, 1996). In the past there has been considerable debate over which of the above pathways is of greater significance in terms of metal exposure in molluscs. However, recently there has also been a growing acceptance that both pathways must be resolved to avoid underestimating full exposure (Luoma, 1996). There are additional means for contaminant uptake including pinocytosis of metal-rich particles and mixing of the

external medium directly with body fluids. However, due to their minimal contribution to body burdens (Phillips and Rainbow, 1993), these mechanisms will not be discussed.

The route of metal exposure in molluscs appears to vary with metal species as well as with biological factors pertaining to the food source (particle concentration, nutritional quality, presence of organic coatings) and assimilation efficiencies of the metal by individual mollusc species. It is therefore more advantageous to examine both routes of uptake on a metal by metal basis and the factors that control the bioavailability of metals to mollusc through both exposure routes. Before discussing the bioavailability of metals from both exposure routes, some laboratory and field data are presented showing the relative dependence of different metals on each pathway and the reasoning behind the data.

3.5.2.1 Sources of metal exposure. The route of uptake for an individual metal species will depend on its particular binding affinities for specific ligands. Metal ions that have Class B or borderline characteristics (Nieboer and Richardson, 1980) have high affinities for sulfur and nitrogen ligands and as a result bind to proteins and other cellular macromolecules. This high affinity for proteins causes the metals to bind to transport-proteins (i.e. intrinsic proteins) which then traverse the plasma membrane and transfer the metals to ligands of higher affinity within the cell (Phillips and Rainbow, 1993). Ag, Zn, and Cd bind strongly with sulfur groups in protein and show the greatest propensity for carrier protein transport across membranes. In some instances, Class B or borderline metal ions are taken up by "accident" by transport systems designed for other major ions due to similar ionic characteristics. There is evidence for the transport of the Cd ion using a Ca channel (Phillips and Rainbow, 1993). The high demands of molluscs for Ca have been related to atypical high Ca pump activities which may enhance Cd uptake to the point where it becomes the primary route (Phillips and Rainbow, 1993). Comparison of labeled Cd and Ca in the Australian freshwater mussel *Velesunio angasi* showed a strong correlation between the two ions (Phillips and Rainbow, 1993). The metal Mo, which occurs in seawater predominantly as an oxygenated anion, uses active transport pumps specific for the metal ion or those designed for the uptake of other anions (sulfate,

phosphate) (Phillips and Rainbow, 1993). Neutral metal complexes (e.g. $HgCl_2^0$, $CdCl_2^0$) and organometallic compounds (e.g. CH_3HgX) are lipophilic and may be directly transported across the hydrophobic plasma membrane by passive diffusion (Phillips and Rainbow, 1993).

Tessier et al. (1984) suggest that the high levels of Cu, Zn and Pb associated with the gill and mantle and the high contribution of those tissues to the body burden indicates that dissolved trace metals are an important route of uptake for *Elliptio complanata*. Nevertheless, the authors also suggest that direct uptake of metal particles by the gills and mantle by endocytosis is possible.

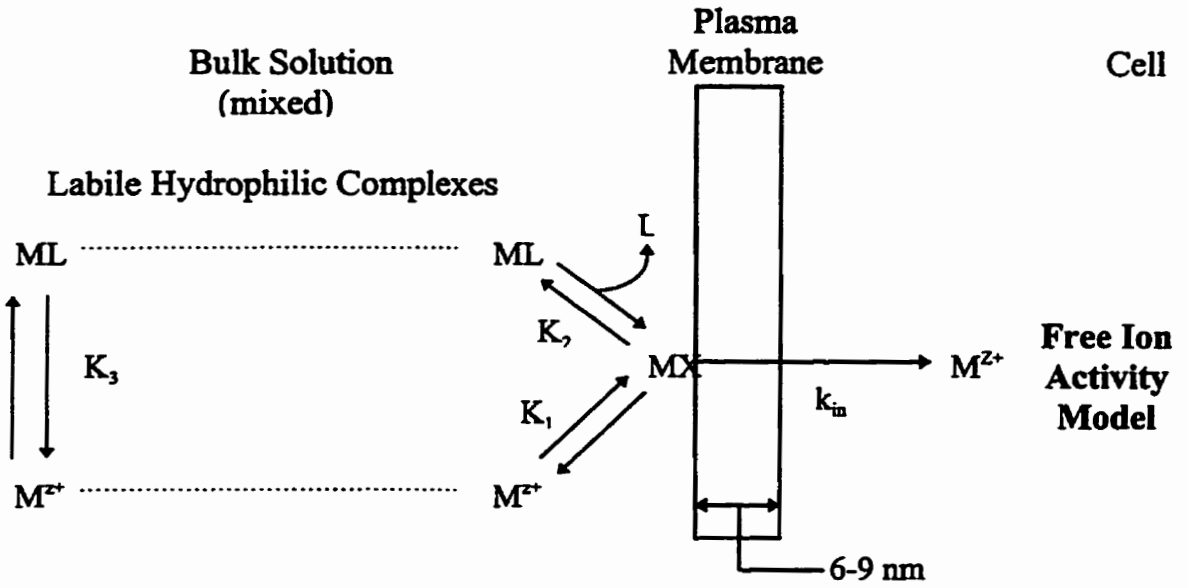
Wang et al. (1996) found that trace elements vary widely in their accumulation patterns in marine mussels (*Mytilus edulis*) and that both dissolved and particulate metals are appreciably accumulated. The relative importance of each source is principally related to metal partitioning on food particles, metal assimilation efficiency and to a lesser degree, the seston load. Based on a model, Wang et al. (1996) predicted the primary route of uptake under varying environmental conditions (seston load, K_d) to be >50-80% for Cd and 30-70% Ag from water and 50-72% for Zn from food.

3.5.2.2 Dissolved phase. Considerable evidence suggests that the total aqueous concentration of a metal is not a good predictor of its bioavailability (Campbell and Tessier, 1996). Metal speciation greatly affects metal availability to aquatic organisms; only a portion of the total dissolved metal is bioavailable. Of the dissolved metal species, the free metal ion concentration $M^{z+}(H_2O)_n$ has been shown to be linked to biological responses (Campbell and Tessier, 1996). The free metal ion concentration is a function of both the total aqueous metal present and the quantity and nature of ligands present in the water. Consequently, it is not surprising that the free metal ion concentration can vary widely among systems as does the biological response it causes. The free-ion activity model (FIAM) developed by Morel (1983) and reviewed recently by Campbell (1995) has been relatively successful in predicting the bioavailability of dissolved metals that exist in natural waters.

The major concepts of the FIAM will be presented here, but for more detailed description of the underlying equations of the FIAM the reader is directed to Campbell (1995). The FIAM is essentially based on the premise that in a system at equilibrium, the free-metal ion activity reflects the chemical reactivity of the metal (Fig. 3.2). The reactivity of the metal determines the extent of the reaction of the metal with surface cellular sites, and hence its bioavailability. In order for a metal to be accumulated by an organism, a metal must first interact with and/or cross a cell membrane (Campbell and Tessier, 1996). This interaction takes place with the free metal ion (M^{z+}) or a metal-ligand complex (ML^{z+}) as the reactive species, and results in the formation of M-X-cell surface complex, where X-cell is a cellular ligand present at the cell surface. Assuming the concentration of free -X-cell sites remains approximately constant, the biological response (accumulation, toxicity, etc.) will vary as a function of $[M^{z+}]$. In the case where a metal complex (ML^{z+}) reacts at the cell surface, the reaction must be accompanied by the loss of ligand "L" (i.e. ML + X-cell ⇌ M-X-cell + L, where charges on individual species are not shown). Thus, the idea that the free hydrated metal ion is the only bioavailable species is a misconception, since no single species in a solution can be considered more (or less) available than another (Campbell, 1995). There are a number of key assumptions surrounding the FIAM involving the biological surface and the kinetics of metal-organism interactions (Table 3.6).

If the dissolved phase is the primary exposure vector, the metal concentrations in the molluscs should be correlated with the free-metal ion concentration in the ambient water $[M^{z+}]$ or its surrogate. The review by Campbell and Tessier (1996) of bioassays and field surveys of indigenous benthic organisms supports the idea that benthic organisms respond to the free-metal ion concentration in the ambient water in or near the surficial sediments (Table 3.7).

For field studies, the geochemical gradient (metal bioavailability) has been defined in terms of the free-metal ion (as estimated from sediment-water equilibria) or the ratio of sorbed metal to sorbent (related to the free-metal ion concentration),



M^{z+} = free metal ion; L = ligand; ML = metal complex in solution; M-X-cell = surface metal complex; K_1 and K_2 = equilibrium constants for formation of the surface complex; k_{in} = kinetic rate constant for internalization or transport of the metal across the biological membrane; K_3 represents the complexation reaction in solution (M+L ⇌ ML).

Figure 3.2. Concept of the Free Ion Activity Model (FIAM). Redrawn from Couillard (1996).

$$[M^{z+}] = \frac{\{Fe-OM\}}{K_{M-Fe}[Fe-ox]}$$

where, Fe-ox is the measured concentration of amorphous iron oxyhydroxide and Fe-OM is the measured concentration of metal "M" coextracted with the Fe-ox sorbent. Field-derived equilibrium constants ($K_{M\text{-}Fe}$) for the sorption of trace metals on amorphous Fe (III) oxyhydroxides are pH-dependent - see Table 3.8.

Table 3.6. Assumptions of the free-ion activity model (FIAM). Adapted from Campbell and Tessier (1996).

Biological surface:

- The key interaction of a metal with a living organism involves the plasma membrane, which is impermeable to the free metal ion, M^{z+}, and to its (hydrophilic) complexes, $ML^{\pm}$.
- The interaction of the metal with the plasma membrane can be described as a surface complexation reaction, forming M^{z+}-X-cell ($M^{z+} + \bar{X}$-cell $\xleftrightarrow{K_{ad}}$ M^{z+} - X-cell) (K_{ad} is an apparent (concentration) equilibrium constants). The biological response, whether it be metal uptake, nutrition, or toxicity, is proportional to the concentration of this surface complex; variations in $\{M^{z+}$-X-cell$\}$ follow those of $[M^{z+}]$ in solution ($\{M^{z+}$ - X-cell$\} \leftrightarrow K_{ad}\{\bar{X}$-cell$\}[M^{z+}]$).
- The biological surface does not change during the metal exposure experiment (i.e., the FIAM will be more applicable to short-term experiments than to long-term chronic exposure).

Kinetics:

- Metal transport in solution, towards the membrane, and the subsequent surface complexation reaction occur rapidly, such that a (pseudo-) equilibrium is established between metal species in solution and those at the biological surface ("rapid" = faster than metal uptake, faster than the expression of the biological response).
- Thus, the identity of the metal form(s) reacting with the plasma membrane is of no biological significance. No one species in solution can be considered more (or less) available than another.

Table 3.7. Relationship between metal bioavailability, as determined on indigenous molluscs, and geochemical estimates of the free-metal ion concentration present in the oxic sediment-interstitial water. Adapted from Campbell and Tessier (1996).

Metal	Mollusc	Geochemical predictor	Site	Reference
Cd	filter-feeder *Anodonta grandis*	$[Cd^{2+}]$, estimated from oxic sediment-water equilibria	Rouyn-Noranda; Chibougamau; Eastern Townships, Quebec; Sudbury; Muskoka (N=19); r^2=0.82; p<.01	Tessier et al., 1993
Cu	filter-feeding *Elliptio complanata*	{Fe-Cu}/{Fe-ox}, both extracted with NH_2OH•HCl	Rouyn-Noranda(N=8; r^2=0.95; p<.01)	Tessier et al., 1984
Pb	estuarine deposit feeder *Scrobicularia plana*	{Fe-OPb}/{Fe-ox}, both extracted with HCl	U.K estuaries (N=37; r^2=0.88; p<.01)	Luoma and Bryan, 1978
Hg	estuarine deposit feeder *Scrobicularia plana*	{Hg}/{OM}, Hg extracted with HNO_3; organic matter by loss on ignition	U.K estuaries (N=78; r^2=0.63, p<.01)	Langston, 1982
As	estuarine deposit feeder *Scorbicularia plana*	{Fe-OAs}/{Fe-ox}, both extracted with HCl	U.K estuaries (N=75; r^2=0.93; p<.01)	Langston, 1980

Table 3.8. Field-derived equilibrium constants for the sorption of trace metals on amorphous Fe (III) Oxyhydroxides.

Metal	Relation	
Cd	$\text{Log } K_{Fe\text{-}Cd} = 1.03\ pH - 2.44$	($r^2 = 0.80$; n=26)
Cu	$\text{Log } K_{Fe\text{-}Cu} = 0.64\ pH + 0.10$	($r^2 = 0.75$; n=39)
Ni	$\text{Log } K_{Fe\text{-}Ni} = 1.04\ pH - 2.29$	($r^2 = 0.87$; n=29)
Pb	$\text{Log } K_{Fe\text{-}Pb} = 0.81\ pH + 0.67$	($r^2 = 0.81$; n=7)
Zn	$\text{Log } K_{Fe\text{-}Zn} = 1.21\ pH - 2.83$	($r^2 = 0.89$; n=41)

From Tessier, A., 1992. In: Environmental Particles - Environmental, Analytical and Physical Chemistry Series, edited by J. Buffle and H. P. Van Leeuwen, Lewis Publishers, Boca Raton, FL, pp. 425-453.

Two approaches can be used to estimate the aqueous concentration of metals $[M]_i$ depending on the redox potential of the medium (Campbell and Tessier, 1996). Under oxic conditions $[M]_i$ is controlled by sorption reactions on such sorbents as Fe- or Mn-oxyhydroxides or sedimentary organic matter. Alternatively, under anoxic conditions $[M]_i$ is controlled by precipitation-dissolution reactions with reactive amorphous sulfides (Acid Volatile Sulfides, or AVS). For a more complete description of these types of approaches see Campbell and Tessier (1996) and the December 1996 issue of Environmental Toxicology and Chemistry. Determining which of the two approaches is more appropriate depends on the site in question. Molluscs are almost exclusively in contact with the oxic layer since the water they filter is above or near the sediment-water interface, therefore, sorption reactions in oxic sediments are presumably more relevant in determining $[M]_I$ available to molluscs. It is likely that reactions with AVS may control a portion of the metal available to molluscs; however, field studies on a variety of sites are needed to test the validity of this statement.

3.5.2.3 Particulate phase. The bioavailability of metals primarily obtained in particulate form is determined by factors controlling assimilation efficiencies such as particle type and size ingested and digestion chemistry in the gut (pH, pE, residence time). Freshwater bivalves appear to utilize only particles less than 80 μm and have a maximum retention efficiency of particles in the 5 to 35 μm range (see section 3.4.3.2). The amount of metal associated with these particles generally increases as their size decreases (i.e. increased surface area for binding). Digestive processes may also affect the fate of ingested sediment-bound metals. Metals may be assimilated from particles after their release from particles in the gut (Luoma, 1983). Gut pH levels tend to vary from a pH of 5 recorded for suspension feeders such as oysters to a pH of 6 to 7 for deposit feeding organisms (Luoma, 1983). Uptake in the gut would be expected to increase due to the desorption expected at a low pH, except the concomitant increase in $[H^+]$ competes for available uptake sites on the intestinal membrane (Campbell and Stokes, 1985). Further, the membrane carriers across the gut are reportedly less efficient in complexing the metal for transport than membrane carriers in other tissues such as the gill (Luoma, 1983).

Long residence times of food particles in the digestive tract increase the potential for metals to be assimilated, particularly for those metals with long desorption/dissolution rates (Wang and Fisher, 1996a). Long residence times also improve the extraction efficiency of the metals from the particles. For many bivalves (after food is sorted in the stomach), digestion can be a two-step process. Initially, food particles undergo "rapid" intestinal digestion whereby enzymatically degraded solute or colloidal organic material and associated metals may be absorbed across the stomach and intestine. Low metal absorption efficiencies are usually associated with intestinal digestion (Decho and Luoma, 1991). Finer particles are then sorted from the solutes and transferred the digestive gland for further intracellular glandular processing called glandular digestion (Decho and Luoma, 1991). During this process, digestive cells phagocytize the particles and digest them intracellularly. The relative time spent in either intestinal (extracellular) or glandular (intracellular) digestion has significant implications for the relative assimilation efficiency of metals associated with ingested food particles. A recent study by Decho and Luoma (1996) found that *Potamocorbula amurensis* reduced its

assimilation efficiency (81 to 51 %) of Cr(III)-labeled bacteria at high Cr(III) concentrations in part by reducing the proportion of bacteria processed by glandular digestion. The relative absorption efficiencies during both intestinal and glandular digestion vary among metals and food particles (e.g. bacteria, diatoms), and can vary between bivalve species (Decho and Luoma, 1996).

Endocytosis is another form of solid phase metal uptake in benthic organisms that involves metal-bearing particles being engulfed by specific amoebocytes and/or digestive vesicles outside the cell membrane in the gut lumen or even outside the gills, forming vesicles that move into the tissues (Luoma, 1983). Digestion within the metal-bearing vesicles that leads to the release of metals into the cytosol is poorly understood, although processes similar to those present in the intestinal tract may exist. The importance of this form of endocytosis as a route of uptake for molluscs is unknown. Further research into the mobilization of the metals from the vesicles is needed since a metal is not truly assimilated until it crosses the vesicular membrane into the cytosol.

The type of particle ingested has a significant influence on the bioavailability of the particulate-bound metals. Laboratory techniques using radio-labeled substrates have been used to determine the relative bioavailability of metals bound to different sediment and food particles (Table 3.9). The major conclusion from the sediment experiments was that metal bioavailability varies for each substrate according to the specific binding affinity of the metal for the particle i.e. the stronger the metal binding affinity the less bioavailable the metal (Campbell and Tessier, 1996). Further, the degree of crystallinity played an important role in determining the bioavailability of the different metals; metals associated with older more highly crystalline solid phases tended to be less bioavailable.

Particle coatings also influence the bioavailability of the sediment-bound metals, and again the effect is dependent on the specific metal. Adherent bacteria and bacterial exopolymer (used in bacterial adhesion) had variable effects on the different metals. The exopolymer adsorbed onto the Fe-ox surface resulted in an increase in the availability of particulate-bound metals in the order Ag>Cd>Zn. Adherent bacteria had no effect on metal uptake by *Macoma balthica* (Harvey and Luoma, 1985a). The role of the exopolymer on uptake and availability was thought to be to stimulate enzymes in the digestive tract that enhanced the removal of the metals from the sediment particles

(Harvey and Luoma, 1985b). Other organic coatings consisting of humic and fulvic acids also influenced uptake and bioavailability. Humic coatings only slightly enhanced Cr bioavailability over non-living particles to the marine suspension feeder *Potamocorbula amurensis* and deposit feeder *Macoma balthica* (Decho and Luoma, 1994). Assimilation of Cd was generally higher than for Cr in both bivalves, but the presence of organic coatings on particles reduced Cd bioavailability compared to uncoated particles (Decho and Luoma, 1994).

The bioavailability of metals from food particles was found to differ among phytoplankton taxa and the cytoplasmic partitioning of metals within the algae. The effect of food composition on metal assimilation in *Mytilus edulis* was recently examined by Wang and Fisher (1996a). *Mytilus edulis* were exposed in the laboratory to radio-labeled algae (2 diatoms, 2 dinoflagellates, 2 chlorophytes, 1 prasinophyte) and glass beads (representing extreme end member of inorganic particles). Chromium was not efficiently assimilated from phytoplankton and over 98% was lost from the mussels within 24 h. Assimilation efficiencies were generally lower for Cd and Zn from chlorophytes *Chlorella autotrophica* and *Nannochloris atomus* compared to other algae, but this trend was not observed for Ag, Am, Co or Cr. The cell walls of chlorophytes are more rigid and resistant to enzymatic digestion and physical breakdown (Atkinson et al. 1972). Therefore, the assimilation of essential metals (e.g. Zn) which normally penetrate the cytoplasm of algae with rigid cell walls (Reinfelder and Fisher, 1991) would be expected to decrease. Non-essential elements such as Cd mostly adsorb onto cell surfaces (Reinfelder and Fisher, 1991), suggesting a different mechanism was operating to cause the reduced assimilation of Cd from the chlorophytes. Gut passage times for metals (Am, Cr, Cd) determined in this experiment suggest that extracellular digestion may be responsible for differences in assimilation among food types. The effect of food type was most significant for Ag which showed a 10-fold difference in assimilation efficiencies among algal species. Assimilation efficiencies were significantly correlated with metal cytoplasmic distribution in algal cells (i.e. % penetration into the algal cytoplasm) for Am and Co but not for Ag, Cd and Zn (Wang and Fisher, 1996a). Assimilation efficiencies for metals adsorbed to glass beads were found to be similar to those for algal diets, except

Table 3.9. Assimilation of sediment- and algal-bound metals by the clam *Macoma balthica* and mussel *Mytilus edulis*. Adapted from Campbell and Tessier (1996).

Metal	Species	Relative Bioavailability Sequence	Reference
Cd	*Macoma balthica*	uncoated Fe-ox » coated Fe-ox, organic detritus	Luoma and Jenne, 1976
Ag	*Macoma balthica*	calcite > Mn-ox » biogenic $CaCO_3$ > Fe-ox > detritus	Luoma and Jenne, 1977
Zn	*Macoma balthica*	biogenic $CaCO_3$ > detritus > calcite > Fe-ox, Mn-ox	
Co	*Macoma balthica*	biogenic $CaCO_3$ > calcite ~ detritus > Fe-ox > Mn-ox	
Cd	*Macoma balthica*	(exopolymer + Fe-ox) > (bacteria + Fe-ox) ~ uncoated Fe-ox natural sediment » alkaline extracted sediment extracted sediment + exopolymer ~ original sediment	Harvey and Luoma, 1985b
Zn	*Macoma balthica*	(exopolymer + Fe-ox) slightly > uncoated Fe-ox natural sediment » alkaline extracted sediment extracted sediment + exopolymer ~ orignial sediment	
Cr (VI)	*Mytilus edulis*	phytoplankton > oxidized marine sediment (2% loss on ignition)	Wang et al. 1997
Cd	*Mytilus edulis*	diatoms ~ dinoflagellates > prasinophytes ~ inorganic particle > chlorophytes	Wang and Fisher 1996a
Zn	*Mytilus edulis*	diatoms > dinoflagellates > prasinophytes ~ inorganic particle > chlorophytes (*Nannochloris atomus*)	
Ag	*Mytilus edulis*	dinoflagellates > chlorophtyes > diatoms » prasinophytes	

for Co which more efficiently assimilated from glass beads. In another experiment by Wang and Fisher (1996b), diatom protein content was not found to have a major influence on metal assimilation in *Mytilus edulis*.

Given the differences in metal bioavailability and assimilation efficiency among sediment and food particles, the composition of ingested particles should be considered when interpreting metal accumulation data and estimating the potential for metal bioaccumulation at mining sites.

3.5.2.4 Measuring bioavailability - Practical considerations. Countless field studies have endeavored to relate tissue metal concentrations to total metal concentrations in the sediment or water with limited success. In many cases, cost-cutting measures and time limitations have lead to the use of less complicated measurements of total metals in water and sediment. However, as the above sections illustrate, the amount of metal available for uptake varies as a function of a number of environmental variables and, thus, total metal concentrations are often misleading. Some investigators have successfully related tissue metal concentrations to total metal concentrations in sediment. For example, concentrations of Zn in *Elliptio complanata* and Cd, Cr and As in *Lampsilis radiata* and *Elliptio complanata*, were significantly correlated with concentrations of these metals in the sediment (readily extractable or total) (Metcalfe-Smith et al. 1992). Correlations were positive for Cd, Cr, and As and negative for Zn. Cadmium levels in caged *Lampsilis ventricosa* used to monitor pollution from mine tailings in the Big River, Missouri, were highly correlated with total concentrations in sediment (r=+0.89) (Czarnezki, 1987).

Total metal concentrations in the environment might be expected to provide a reasonable estimate of bioavailable metals for the following cases:

- non-essential metals
- over wide ranges of total metal concentrations (i.e. including grossly contaminated and pristine environments).

- over narrow ranges of underlying geochemistry/geology (e.g. for lakes on the Canadian Shield).

In a more general context, it is strongly recommended that an effort be made to collect environmental data (sediment and water) such that detailed estimates of bioavailable metals can be made. The bioavailable fraction in water for most metals can be estimated from water chemistry and surficial sediment concentrations (see Tessier et al., 1993).

3.5.2.5 Summary.

1. Bioavailability estimates should consider metal contributions from both routes of uptake (dissolved and particulate) and should be determined for individual metals.
2. The uptake from the dissolved phase can be estimated using the Free Ion Activity Model. The free-metal ion concentration can be estimated from sediment-water equilibria. Variations in the relative values of the free-metal concentration can be approximated by the ratio of sorbed metal to sorbent, provided that the pH is reasonably constant.
3. The bioavailability of metals from the particulate phase is determined by factors specific to sediment and food particles. Metal assimilation from sediment particles is influenced by chemical composition (Fe-ox, calcite etc.) and presence of organic or bacterial coatings. Differences in metal assimilation from algae result from the relative amount of time spent in extracellular and intracellular digestion, algal cytoplasmic metal distribution and cell wall structure.

3.5.3 Biological characteristics

There is considerable evidence that individual biological characteristics affect metal accumulation in molluscs. This is not surprising since many metals are essential for growth and reproduction and would be accumulated in varying amounts depending on the biological state of the individual mollusc. Aspects of age, size and growth rate, sex and reproductive status, behavior and inter-specific differences and how they affect metal accumulation are briefly addressed below. In addition, metal accumulation in

transplanted or caged molluscs will be compared to that in indigenous populations. Decisions about the usefulness of transplanted or caged molluscs in monitoring programs depend in part on their ability to mimic metal accumulation of indigenous populations from impacted areas.

3.5.3.1 Age, size and growth rate. The effect of age and size was followed by growth rate and then sex in order of importance in predicting tissue metal concentrations (Cd, Cu, Fe, Hg, Mn, Ni, and Zn) in the freshwater bivalves *Elliptio complanata* and *Lampsilis radiata radiata* collected from the Sorel delta in the St. Lawrence River (Metcalfe et al. 1996). Levels of As, Cd, Mn, Zn, Hg, and Fe were higher in older-larger individuals of both species, whereas Cu, Al, Ni, Cr, and Se were higher in younger, smaller individuals of *Lampsilis radiata radiata*. In *Elliptio complanata*, only Cu was higher in younger, smaller individuals. Lead levels were not affected by age or size in either species (Metcalfe-Smith et al. 1996). In the freshwater prosobranch *Bithynia tentaculata* collected from Lake St. Louis and St. Pierre in Quebec concentrations of Cu and Zn were significantly higher in adults compared to juveniles, whereas no life-stage differences were found for Cd, Pb or Ni (Flessas et al., submitted). Age and size successfully predicted tissue metal concentrations in *Elliptio complanata* collected from an acid-sensitive and circumneutral lake in Ontario, in metal- and tissue-specific manner (Hinch and Stephenson, 1987). For example, mussel age was a better predictor of gill Zn and Mn concentrations, but gill Cd was better predicted by mussel size.
However, the age and size of individuals are not always correlated (Hinch and Stephenson, 1987). Relationships between metal concentrations and size can vary among metals, among species, and among studies (Table 3.10). The overall effect of age and size on metal concentrations in molluscs also depends on growth rates, feeding habits and reproductive status. For example, Malley et al. (1989) found that growth rates were distinctly different between two populations of *Pyganodon grandis* exposed *in situ* to ^{109}Cd in a Precambrian Shield lake over the ice-free season. The smaller, slower growing bivalves had higher concentrations of Cd in their tissues compared to the larger, faster, growing bivalves of the same age. The incorporation of Cd into the tissues of bivalves is

linked to their filtration rates and metabolism. Thus, even though the faster growing individuals probably accumulated metals at a faster rate, their large body size diluted the actual metal concentration. Because it is difficult to predict the effect of age and size, it is recommended that age and size either be standardized in all monitoring programs or tested for age- and size-specific differences prior to grouping specimens together (Hinch and Stephenson, 1987).

3.5.3.2 Sex and reproductive status. The sex and reproductive status of molluscs may influence tissue metal concentrations in bivalves. Differences in tissue metal concentrations of male and female bivalves collected from a range of contaminated sites along the St. Lawrence River were significant in both *Lampsilis radiata radiata* (Cd, Fe and Zn were higher in males), and *Elliptio complanata* (Cu was higher in females) (Metcalfe-Smith, 1994). Males tended to display less variability in metal concentrations than females. The effect of sex on tissue metal concentrations was less significant than inter-species differences.

Lobel et al. (1989) found that in "post-spawn" *Mytilus edulis* collected from Bellevue, Newfoundland As, Cu, Mn and Zn were higher in females, Pb was higher in males and Al and Cd did not differ between sexes. Alternatively, Jones and Walker (1979) found no sex-specific differences in metal tissue levels in freshwater *Velesunio ambiguus* from the River Murray in South Australia.

In general, tissue concentrations of metals in filter-feeding bivalves are highest in males and females just prior to spawning and differences between sexes are minimal at this time compared to other times throughout the year. For biomonitoring programs, Lobel et al. (1991a) suggest that the sexes be analyzed separately and that the sampling be performed prior to spawning to reduce individual differences.

3.5.3.3 Behavior. Recently developed laboratory techniques reveal that unionids may close their valves in response to high metal concentrations (Kramer et al., 1989; Doherty et al., 1987). Kramer et al. (1989) found that *Mytilus edulis* elicited a valve closure

Table 3.10. Relationship between metal concentrations and size and age (inferred from size) in marine molluscs: metal concentrations increase with size (+), decrease with size (-), or are not related to size (0). Adapted from Hinch and Stephenson (1987).

Species	Trace metal	Metal-size relationship	Reference
Mercenaria mercenaria	Cu	+	Romeril, 1979
	Zn	+	Romeril, 1979
	Mn, Zn	-	Boyden, 1977
Mytilus edulis	Cu, Cd, Zn	o	Brix and Lyngby, 1985
	Cd, Mn, Zn	+	Szefer and Szefer, 1985
	Cu	-	Szefer and Szefer, 1985
	Cu	-	Popham and D"Auria, 1983
	Cd	+	Ritz et al., 1982
	Zn	o	Ritz et al., 1982
	Cu	-	Ritz et al., 1982
	Cu, Cd, Zn, Mn	-	Cossa et al., 1980
	Cd, Zn	+	Harris et al.,1979
	Mn, Cu	-	Harris et al., 1979
	Cd	o	Boyden, 1977
Ostrea edulis	Mn	+	Boyden, 1977
	Zn, Cu, Cd	o	Boyden, 1977
Patella vulgata	Cd	+	Boyden, 1977
	Zn	-	Boyden, 1977

response when exposed to 38 μg L^{-1} Cu (as copper sulfate). At lower Cu concentrations (<20 μg L^{-1}) the closure response was delayed and less obvious. Similar detection limits, determined when 7 or more out of 10 mussels reacted by closing their valves or changing filtering activity, for Cd (<100 μg L^{-1}), Cu (<10 μg L^{-1}), Zn (<500 μg L^{-1}) and Pb (<500 μg L^{-1}) were found for *Mytilus edulis* and *Dreissena polymorpha* (Kramer et al., 1989). The metal concentrations used in the above study and those used by Doherty et al. (1987) (100 to 400 μg Cd L^{-1}) were well above typical environmental metal concentrations and thus it is difficult to determine their relevance for mollusc biomonitoring studies. If unionids can limit the uptake of metals during periods of shell closure, metal levels in unionid tissues may be underestimated. Further research on the influence of valve closure on metal accumulation at environmentally realistic concentrations is needed.

3.5.3.4 Inter-specific differences. The advantages of using more than one species in a biomonitoring program include broadening the geographic range and increasing the habitat coverage (Metcalfe-Smith, 1994). However, differences in metal accumulation among freshwater and marine bivalve species have been reported and may limit inter-species comparisions. Metcalfe-Smith (1994) found that in freshwater mussels collected from a range of contaminated sites, *Elliptio complanata* accumulated significantly more Al, Cr, Fe, Hg and Ni and less As, Cu, Mn and Zn than the mussel *Lampsilis radiata radiata*. There were no inter-species differences for Cd and Pb, except for Cd when the sexes were separated. *Elliptio complanata* tended to show a broader range of responses across contaminated sites than *Lampsilis radiata radiata* suggesting that the latter species may be more capable of regulating tissue metal concentrations. This would make *Lampsilis radiata radiata* less suitable as a biomonitor since it less directly reflects the bioavailability of metals in the environment (Metcalfe-Smith, 1994).

Differences among species were generally in the range of 1.2 to 2.5 X which corresponded to other similar studies (Metcalfe-Smith, 1994; Metcalfe-Smith et al. 1992). Although these differences don't appear to be large, if differences among study sites only range between 2 to 10X, the ability to use different species to distinguish among sites diminishes (Metcalfe-Smith, 1994). Tessier et al. (1987) report higher metal concentrations (Cd, 1.7 - 3.0 X; Cu, 1.1 - 2.3 X; Pb, 0.9 - 8.3 X; Zn, 1.2 - 1.9 X) in indigenous *Pyganodon grandis* compared to indigenous *Elliptio complanata* in both Lake Memphrémagog, Quebec and Lake St. Nora, Ontario. A similar range of differences among species were found for two marine mussels collected from a lagoon in Newfoundland whereby concentrations of all the 25 elements analyzed (except Mn) were higher (1.5X) in *Mytilus trossulus* than in *Mytilus edulis* (Lobel et al. 1990).

Despite inter-species differences in absolute metal concentrations, some metals in Metcalfe-Smith (1994) were highly significantly correlated (Al, Cd, Cu, Fe and Pb) among the species while others were significantly correlated (Cr, Hg and Ni). Therefore, Metcalfe-Smith (1994) suggested that *Elliptio complanata* and *Lampsilis radiata radiata* could be used interchangably to monitor site-to-site trends in the bioavailability of certain metals (e.g. Cd and Pb). Trends in other metals (Al, Cr, Cu, Fe, Hg and Ni) could also be compared between the two species, but would require a conversion factor developed from

a series of regression equations. Where correlations were lacking (As, Mn and Zn), the two species could not be used interchangeably and further research would be required prior to choosing the better species for monitoring those metals. Nevertheless, relative differences in metal concentrations within one species are often more important in biomonitoring programs than absolute differences. Biomonitoring programs should be carefully designed with the knowledge of inter-species differences in mind, ensuring that there will be sufficient availability of the species for the duration of the monitoring program.

3.5.3.5 Transplanted/caged vs. indigenous populations. Transplanted and/or caged molluscs are often used in metal accumulation studies as representatives of indigenous populations. However, metal accumulation patterns in these experimental animals may not reflect those of the free-living indigenous populations. There are two primary questions relevant to the use of transplanted and/or caged molluscs in monitoring programs: (i) do molluscs that are caged in their "source" lake grow and accumulate metals at the same rate as do the free-living molluscs in the same lake, and; (ii) do molluscs that are moved from an uncontaminated environment (whether it be a control lake or an aquaculture facility) to a contaminated environment grow and accumulate metals at the same rate as do the free-living molluscs in the contaminated environment?

Studies by Couillard et al. (1995a,b) suggest that caging molluscs on the sediment in their source lake does not significantly influence metal accumulation patterns. No significant differences were found between *Pyganodon grandis* caged for 400 days in its source lake and free-living individuals outside the cages with regards to tissue Cd, Cu and Zn concentrations (except Zn in digestive gland), condition index and MT (except mantle MT). However, shell growth was significantly reduced in 400-day caged source lake mussels in both the uncontaminated and contaminated lake, which may have had consequences for metal accumulation if the exposure had continued. No significant differences between individuals caged on the sediment for 7 days and those collected outside the cages were found for Zn and Cd concentrations (except Zn in foot and Cd in digestive gland) in the freshwater clam *Amblema plicata*, near an electroplating plant on

an Indiana stream (Adams et al. 1981). Similarly, Malley et al. (1996) found that total mercury and methyl mercury concentrations and body burdens in tissues of *Pyganodon grandis* caged on the sediment for 88 days in their source lake were not significantly different from free-living mussels outside the cages.

Transplanted molluscs may grow and accumulate metals at the same rate as do the free-living molluscs in the destination lake depending on the metal. Cadmium concentrations in tissues and whole body of *Pyganodon grandis* transplanted from uncontaminated Lake Opasatica to contaminated Lake Vaudray and caged for 400 days were only one third of those found in the free-living indigenous mussel population (Couillard et al. 1995a). In contrast, Zn tissue and whole body concentrations in the transplanted mussels rapidly reached those of the free-living indigenous population (<100 d). Couillard et al. (1995a) suggest that the rapid uptake of Zn and the rapid attainment of steady-state concentrations was consistent with the essential role of this metal. Based on these data, Zn uptake and accumulation in transplanted mussels could be used as representative of that which occurs in indigenous mussel populations. Hinch and Green (1989) found that both the destination and source or collection site influenced growth rates and tissue metal concentrations (Cd, Cu and Zn) in transplanted *Elliptio complanata* after 1 year. Growth rates have been previously reported to be affected by the source of the mussels rather than the transplant destination, suggesting that growth rates may be under direct genetic control (Hinch et al. 1986). To control for the "source effect"in transplant experiments all specimen collections should be from the same site within a lake or from the same aquaculture facility and the exposure periods should exceed 1 year to allow the destination lake to become the dominant influence (Hinch and Green, 1989).

3.5.4 Modeling trace element bioaccumulation in mussels

Recent developments in our knowledge of assimilation efficiencies and uptake rates in freshwater mussels have made it possible to develop models that can be used to estimate bioaccumulation factors (BAFs) to calculate possible concentrations in mussels based on environmental concentrations of trace elements. These models take into consideration the critical environmental variables that influence metal accumulation to make the BAFs more accurate and useful than BCFs. Models offer considerable

advantages for biomonitoring in that bioaccumulation data collected in mussels can be interpreted and the source of metals released into the environment can be better ascertained.

Mytilus edulis fed radioisotope-tagged natural seston or put in a radioisotope-labeled water, was then monitored in a clean environment to measure efflux rates (Wang et al. 1996). Trace metal concentrations predicted using a kinetic model were comparable to concentrations measured in various monitoring programs, suggesting that metal accumulation in mussels can be accurately predicted using the physiological and geochemical parameters identified (C_w, water concentration, C_f, food concentration, Total suspended solids and K_d). Two important physiological parameters, metal absorption efficiency from the dissolved phase and assimilation efficiency from food, must however be known if this approach is to be used to assess metal bioavailability to aquatic animals.

3.6. Relationship between tissue (or whole body) metal concentration and effects in molluscs

Rather than relate metal effects in molluscs to metal concentrations in the sediment and water they will be related to mollusc tissue-residues. This approach tends to improve the relationships drawn between metal exposure and effects because the concentrations of metals in tissues are implicitly bioavailable; as described in the previous section, determinations of "bioavailable metal" can be complicated.

3.6.1 Interpreting tissue-residue effects in molluscs

The relationship between tissue (or whole body) metal concentrations ("Dose") and effects in molluscs is dependent on many factors including the metal (i.e. chemical form, essential vs. non-essential), type of effect (i.e. biochemical, physiological, population), exposure history (e.g. development of tolerant populations), environmental conditions (i.e. temperature, food availability, presence of other stressors) and numerous biological characteristics (i.e. species, age, size and reproductive state). These factors must be well described and understood in order to successfully relate dose to effects in molluscs as well as in any other organisms. Luoma and Carter (1991) suggest that attributing a biological change in a natural system to the specific influences of metals requires: 1) demonstrating which processes are sensitive to metals; 2) separating metal-induced changes in a process from background fluctuations, and; 3) unambiguously relating the detected change to metal exposure rather than abiotic (e.g. temperature, salinity, oxygen, or physical processes) or biotic (e.g. species interactions, nutritional status) confounding variables.

Effects may occur at different levels of biological organization including biochemical, physiological, population and community (Luoma and Carter, 1991). The "effect" or functional impairment within a level occurs after the compensatory capabilities are overcome (Luoma and Carter, 1991). Effects at lower levels of biological organization (biochemical and physiological) do not necessarily result in observable effects at higher levels of organization (population and community). Typically, as the exposure increases the compensatory capabilities are overcome at the higher levels of

organization and changes in the population and community structure results. Effects at the biochemical or physiological level have been recommended for use as early warning signals for effects at higher organizational levels. However, few field studies have attempted to relate effects at the biochemical or physiological level to effects at higher levels of biological organization. Therefore, the ecological relevance of many biochemical or physiological responses is unknown, which severely limits their usefulness in monitoring programs.

The development of effects monitoring in molluscs has benefited from recent collaborative field and laboratory studies. Although effects in molluscs have primarily consisted of laboratory some effects have been successfully identified and quantified in the field. Field studies provide data which are more relevant to biomonitoring programs. Recently there has been a rise in the number of studies that link effects at all levels of biological organization. The effects that have received the most recent attention include changes in growth, condition index, and metallothionein (MT). Changes in MT concentration, although described here as an effect, is actually an indicator of exposure to metals. Molluscs have been included in measurements of community structure (e.g. taxonomic indices), but, the relatively small numbers of species found in Canada render mollusc population measurements not very useful. The following is a brief summary of how growth and MT concentrations can be related to tissue metal concentration in bivalves.

3.6.2 Growth

Growth is a physiological endpoint measured at the individual organism level that reflects the environmental conditions in which the organism lives. Natural environmental conditions such as food quality and quantity and temperature as well as characteristics of the organism (e.g. size, age, sex) are major determining factors of growth in individuals, although metal exposure can play a role. Growth is one of many physiological responses that are sensitive to metals, although it tends not to be metal-specific (Luoma and Carter, 1991). Nonetheless, effects of metal exposure on growth have been well studied (in the

marine environment) and may prove to be a suitable effects measurement as part of a monitoring program.

3.6.2.1. General description. Growth in molluscs can be determined in many different ways including changes in shell length and soft-tissue weight. Changes in absolute growth as well as growth rates of bivalve molluscs can be determined non-destructively using the external rings visible on the shells. It is generally accepted that populations of bivalves in northern climates put down one new external growth ring a year, based on mark and recapture studies (Metcalfe-Smith and Green, 1992 and references therein). A recent study that utilized a similar type of mark and recapture, found that external annual rings were formed less frequently than annually for several *Pyganodon grandis* casting doubt on the process of aging molluscs (Downing et al. 1992). However, the generality of this observation cannot be established at this time without further field studies. There have been numerous unionid species that have been successfully aged using the above technique including *Anodonta piscinalis*, *Anodonta anatina*, *Anodonta grandis simpsionata*, *Elliptio complanata*, *Lamsilis radiata siliquoidea* and *Leptodea fragilis* (Metcalfe-Smith and Green, 1992).

To determine an individual's growth rate external annual growth rings are measured and the total number of growth rings counted. These measurements are then entered into "Walford Plots" to determine the shell growth rate. For example, the length delimited by an annulus for year n+1 is regressed against the length for year n (Fig. 3.3). Comparison of the regression coefficients for bivalve populations can be then compared. Von Bertalanffy growth curves, which relate individual annular length (y-axis) to the age of the bivalve, provide a more qualitative means for comparing relative growth of different bivalve populations (Fig. 3.4). Using both the Walford Plot and the Von Bertalanffy growth curves similarities between bivalve populations can be determined as well as growth potential for a particular year, such as in Couillard et al. (1995a). Growth of individual bivalves can be measured at the beginning and end of exposure periods, providing the marking technique does not cause excessive stress on the animals. A considerable number of studies have been done by Green et al. (1989) and others (Hinch

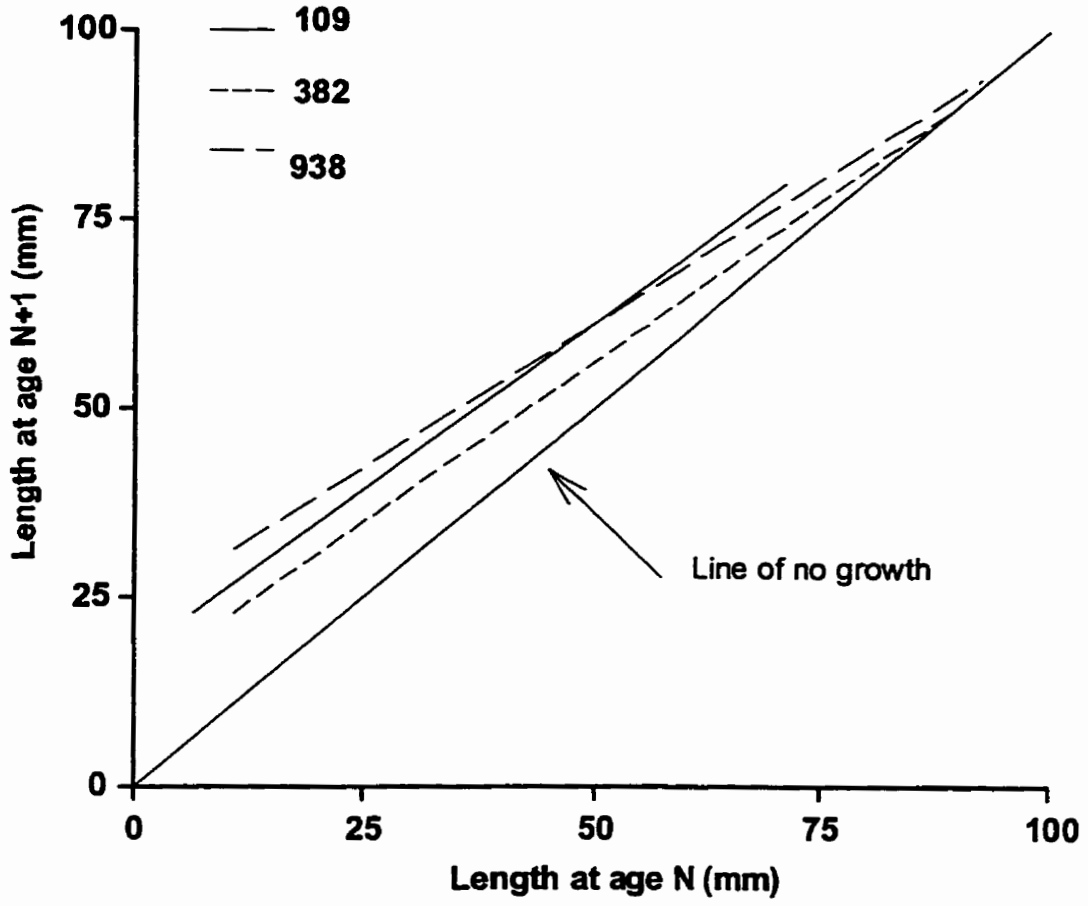


Figure 3.3 Walford plot for *Pyganodon grandis* collected from Lakes 109, 382 and 938 in the Experimental Lakes Area, Northwestern, Ontario (Stewart, unpublished data).

and Green, 1989; Hinch et al. 1986; Hanson et al. 1988) to quantify the influence of natural environmental factors on shell growth. Shell growth must be interpreted with caution since hydrodynamic conditions (i.e. wave action) can influence the allometric growth of the shell and thus indirectly influence growth rate measurements (Green et al. 1989).

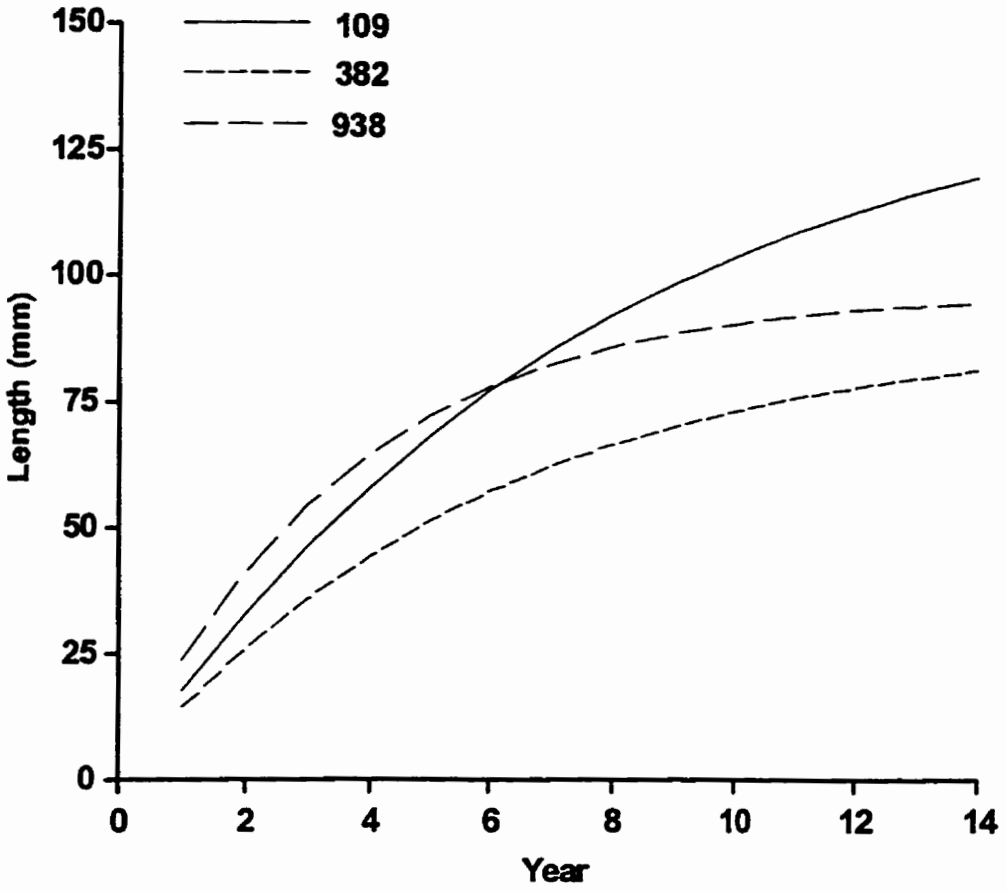


Figure 3.4. Von Bertalanffy growth curves for *Pyganodon grandis* collected from Lakes 109, 382 and 938 in the Experimental Lakes Area, Northwestern, Ontario (Stewart, unpublished data).

Changes in soft-tissue weights are another useful measure of mollusc growth. Measurements of individual tissues or the whole organism can be made on sacrificed individuals and compared among composite samples of pre- and post-exposure groups. Sampling error is reduced by ensuring that the shell cavity is full of water, to maintain a constant water content, and that the shell is free of debris.

3.6.2.2. Dose-Response. There are significant difficulties in attributing growth impairment in bivalve molluscs to metal exposures. Natural cycles in food availability and differences in temperature among sampling sites can confound metal-induced growth effects. All bivalves, caged and native, marine and freshwater, have shown natural fluctuations in dry soft-tissue weights. For example, Cain and Luoma (1990) found a constant decrease in the soft tissue weight in indigenous *Macoma balthica* in San Francisco Bay for two consecutive years. Couillard et al. (1995a) showed decreases in organism dry weights of indigenous and caged populations of *Pyganodon grandis* over a 400 day exposure period, although the decreases were more pronounced in the bivalves exposed to higher metal concentrations. Salazar et al. (1996) found a significant negative linear correlation between tissue mercury concentrations and changes in whole-organism wet-weights of *Elliptio complanata* caged for 84 days along a mercury contamination gradient. A significant negative correlation was also found between soft-tissue wet-weights in *Elliptio complanata*, measured at the end of the study, and tissue mercury concentrations. The overall reduction in growth of *Elliptio complanata* could not be attributed entirely to elevated mercury concentration because of other confounding physico-chemical factors (i.e. temperature).

Relationships between shell growth and metal exposure are also sensitive to environmental conditions. Holding the mussel, *Pyganodon grandis* in open enclosures in both high and low metal contaminated lakes for 400 days had a significant negative effect on growth rate. In the first 90 days mussels reached 75% of their annual growth increments. Couillard et al. (1995a) suggested that the caging effect on growth rate could have been an artifact of the temperature range in which the bivalves were caged. Hanson et al. (1988) found that *P. grandis* caged for over a year at 1 and 3 m grew faster than those caged at 7 or 5 m and that the differences were strongly correlated to temperature at either depth. Couillard et al. (1995a) hypothesized that bivalves left in their natural environment grew faster because they were able to migrate vertically to micro-environments more favorable to their growth. Nevertheless, bivalves caged in the more metal contaminated lake had a significantly lower mean growth rate than bivalves caged in the control lake. Both the Couillard et al. (1995a) and Salazar et al. (1996) studies

compared bivalves from the same population, ensuring that the genetic aspects of growth in the bivalves were held constant and thus, could not confound the results.

Metcalfe-Smith and Green (1992) utilized regression relationships between the bivalve age and shell length and shell weight to compare mussel populations from a low and high As and Hg contaminated lake (bottom sediment: low contaminated - As and Hg <detection; high contaminated - As = 890-3050 $\mu g\ g^{-1}$ dry wt and Hg = 5.7 $\mu g\ g^{-1}$ dry wt.). *Elliptio complanata* ranging in age from 5 to 17 years were found to be longer (shell length) at a given age in the less contaminated lake than the more contaminated lake, although the growth rates of the bivalves did not differ for bivalves 5 years and older. However, regression intercepts were significantly different indicating that the bivalves from the less contaminated lake had faster growth rates during the first 5 years of their life than those from the more contaminated lake. An identical relationship was found when age was used to predict shell weight. Similar measurements on the unionids *Anodonta implicata* and *Alasmidonta undulata* did not reveal any significant differences between lakes. The authors suggest that the lower initial growth rates and sizes of *Elliptio complanata* may have been due to the arsenic contamination. Tissue-metal concentrations would have been required in order to further evaluate the role of metal contamination in the observed differences in growth.

3.6.2.3. Recommendations. The above examples suggest that bivalve growth (shell length, growth rate, soft-tissue weights) could be used to monitor metal-induced effects in the freshwater environment. However, field studies should be designed to demonstrate that the observed changes in bivalve growth are caused by exposure to metals and not by subtle changes in physico-chemical variables (e.g. temperature and food). Until this is done, the results of growth studies may continue to produce confounding results that will not serve the needs of the Regulators and Canadian mining industry.

3.6.3 Metallothionein (MT)

For an excellent review of MT the reader is referred to "Technical evaluation of metallothionein as a biomarker for the mining industry" (Couillard, 1996). Here I will

highlight the author's recommendations and provide a background on MT based on Couillard (1996).

3.6.3.1. General description. Metallothioneins are low molecular weight metal-binding proteins found within the cytosol. These metal-binding proteins normally bind Group IB and IIB metal ions including Cd, Cu, Zn, and occasionally Ag. Metallothioneins serve as a compensatory mechanism in response to metals, play a role in the regulation of essential metals and the detoxification of metals, and they provide a cellular basis for the bioaccumulation of metals.

Couillard (1996) describes two potential major roles for MT in the intracellular metal distribution: 1) MT induction results in the interception and binding of metal ions taken up by the cell, and; 2) MT removes metals from non-thionein ligands that include cellular targets of toxicity; this redistribution onto MT is suggested to represent a rescue function. Cellular toxicity is expected when these roles are not carried out effectively. Alternatively, cellular toxicity is also expected when there is excessive accumulation of metals beyond the binding capacity of available MT, resulting in their binding to other intracellular ligands, a phenomenon termed "spillover" (Couillard, 1996). According to this model, the degree of metal detoxification, as determined by intracellular metal partitioning, is a better indicator of metal-induced stress than the absolute measure of MT (Couillard, 1996).

3.6.3.2. Dose-Response. Case studies reviewed by Couillard (1996) indicated that MT concentrations increased in a dose-dependent manner along metal contamination gradients (Table 3.11). Shifts in intracellular metal partitioning towards the very low-molecular-weight metal complexes, typical of metal-induced stress, were detected in contaminated areas. These biochemical effects were accompanied by deleterious effects at higher levels of biological organization (organ, organism, population). Deleterious effects occurred prior to complete saturation of metal-binding sites by the toxic metal, due to competition for binding sites from essential metals. Thus, the spillover hypothesis based on a complete saturation prior to detection of toxic effects was not strictly

observed. Little information was available describing external factors influencing MT concentrations. Although factors related to the basic biology and physiology of molluscs were found to influence MT concentrations, changes in metal bioavailability were deemed more important as sources of variation (Couillard, 1996).

Couillard (1996) noted that most field studies have not been able to "convincingly demonstrate a mechanistic linkage between biochemical responses and adverse effects at higher levels of biological organization" and "further research is needed." Linkages between the hypothesized metabolic costs associated with the activation of tolerance mechanisms such as MT to metal exposure could not be conclusively made at this time, although further research is again recommended.

3.6.3.3. Recommendations. Couillard (1996) recommends the use of MT as a biomarker of exposure to certain metals (notably Cd, Zn, Cu and Ag), but states that MT requires further development as an effects biomarker. In particular, Couillard (1996) recommends further field studies that demonstrate that there are metabolic costs to MT synthesis and/or that the overwhelming of detoxification mechanisms, including MT, is associated with deleterious effects on the host organism.

Table 3.11. Summary of case studies documenting metallothionein in molluscs. Adapted from Couillard (1996).

Field Site	Species	Tissue	Metal Gradient	Result	Reference in Couillard (1996)
Rouyn-Noranda lakes, Quebec (N=11)	Freshwater mollusc (*Pyganodon grandis*)	gills; whole organism	Cd defined in terms of $[Cd^{2+}]$ at sediment-water interface	MT increased 2.5- to 4-fold in the indigenous populations along the contamination gradient; [MT] correlated with increase in tissue Cd.	Section 4.3.1
Rouyn-Noranda lakes, Quebec (Lake Vaudray exposure period = 400 days)	Freshwater mollusc (*Pyganodon grandis*)	gills; whole organism	Cd defined in terms of $[Cd^{2+}]$ at sediment-water interface	MT increased 2.5- to 4-fold over the first 400 days in molluscs transferred from control lake to highly contaminated Lake Vaudray; increase in tissue [MT] correlated with increase in tissue Cd.	Section 4.3.1
Experimental Lakes Area (ELA), Ont. Lake experimetnally contaminated by Cd over 6 years	Freshwater mollusc (*Pyganodon grandis*)	gills; mantle; foot; kidney; visceral mass	Cd defined in terms of $[M]_d$	All body parts produced MT in response to Cd exposure	Section 4.3.3

Table 3.11. Con't.

Rouyn-Noranda lakes, Quebec	Freshwater mollusc (Pyganodon grandis)	gills	Cd defined in terms of $[Cd^{2+}]$	Overwhelming of the detoxification mechanism including MT (MT levels were very high) was observed along the contamination gradient and appeared to be reproducible under severe metal stress (transplantation experiment). This was associated with toxic effects at cellular-, organ-, individual-, and population levels of biological organization.	Section 4.3.1
San Francisco Bay, California, U.S.A	Marine bivalve (Macoma balthica)	whole organism	Ag, Cu defined in terms of $[M]_d$	Overwhelming of the detoxification mechanism including MT was observed in an indigenous population of *M. balthica*. Links appeared to exist between these biochemical measurement and adverse effects at the organism- and population-levels of organization.	Section 4.3.2

3.7 Field testing

This section covers the important factors that should be considered when organizing field studies and analyzing molluscs for metals. Analytical methods of detection and quantification are generally straightforward for molluscs and a number of sources exist from which to establish QA/QC protocols for biomonitoring programs. A number of protocols exist for field studies using molluscs, but aspects of experimental design depend to a large degree on the goals and objectives of the monitoring program and mine site characteristics.

3.7.1 Experimental Design

Determining the experimental design of any field study is critically important and deserves high priority. A good experimental design can mean the difference between useful and worthless data. Molluscs can be useful tools in biomonitoring programs, but they have certain inherent variables that need to be controlled in order to detect changes resulting from mining activities.

3.7.1.1 Statistical considerations. Finding suitable "reference" sites from which to collect specimens for caging studies or which to compare impacted populations with may be difficult. Malley et al. (1996) also describe problems in statistically comparing mercury concentrations in mussels held in a flooded wetland to a reference wetland given that the mercury concentrations were significantly different between the two sites prior to flooding (or impact). There has been significant development in experimental design and statistical approaches for detecting environmental impacts. Most notably is the BACI (Before/After Control/Impact) developed by Stewart-Oaten et al. (1986) and further developed by Underwood (1992). Basically, the BACI approach compares "changes over time" at the reference site to "changes over time" at impacted sites. The question that is asked is "are the changes occurring at the impacted site due to natural variation or due to the impact?" The BACI approach offers an alternative to direct comparisons between a reference site and an "impact" site. For example, many environmental impact studies

compare populations downstream from an industrial plant to reference populations located upstream from the plant. In many cases, there are natural factors that result in differences between the upstream and downstream populations that may mask the true impact resulting from plant operation. The major improvement of the BACI design by Underwood (1992) was to include an asymmetrical sampling design using a randomly-selected set of reference sites. By including more than one reference site the chances of changes occurring at a reference site in the same direction and same intensity as the impacted site (i.e. canceling out the impact) are significantly reduced. For further information on the BACI design the reader is directed to Stewart-Oaten et al. (1983) and Underwood (1992).

The number of samples that should be collected at different sites and on different sampling dates to provide the maximum amount of statistical power is most often limited by analytical costs. The sampling scheme recommended by Crawford and Luoma (1993) for the United States National Water-Quality Assessment (NAWQA) Program is appropriate for the needs of the mining industry. They suggest that 3 composites of 10 individuals be collected from each sample site and sample period. Although 2 or 3 individuals could provide enough material (5 g wet weight) for tissue analysis, 10 animals are sufficient to account for individual variability.

3.7.1.1 Seasonal/spatial variability. Metal concentrations in molluscs may be affected by the season due to changes in temperature and food availability as well as changes in reproductive status and growth. Controlling for these variables depends on the mollusc species, although certain consistent effects have been documented.

Growth in unionids is generally restricted to the warmer, ice-free months of the year. Therefore, collections are usually restricted to these times if the goal is to measure active uptake in molluscs, such as in transplant studies. Several authors recommend that molluscs be sampled just prior to fertilization, since at this time metal concentrations tend to be highest and no glochidia are present in the gills of unionid species (Metcalfe-Smith et al. 1996; Lobel et al. 1991a; Tessier et al. 1993). Changes in food availability are

difficult to control since it may change on a daily, weekly and monthly scale. The best approach is to sample impacted and reference populations at the same time (within a week) and sample at the same time each year for annual trends (assuming no year to year variability).

Spatial variability is an important factor in mollusc studies due to differences in the hydrodynamic conditions, substrate, and micro-habitat (temperature, food availability and quality etc.) that can affect metal uptake. Mollusc populations have been found to differ in genetic composition over short distances, possibly due to their adaptation to a specific habitat (Green et al. 1985). Consequently, mollusc populations collected from different areas of a lake may have inherently different size-age relationships and/or growth rates which can influence metal accumulation. There are several considerations when controlling for spatial variability:

1. Choose sampling sites for comparisons among indigenous populations that share similar hydrodynamic, trophic status and substrates.
2. In mollusc transplant studies all of the individuals needed for the study should be collected from the same area and preferably from the same population.
3. Collect molluscs for transplant studies from an area with similar hydrodynamic, substrate and trophic status as the site of the study. This reduces the effect of "source" on metal uptake and increases the potential for the transplanted molluscs to mimic metal uptake of resident populations (Hinch and Green, 1989). Alternatively, the effect of source can be reduced by extending the transplant studies for periods longer than 1 year (Hinch and Green, 1989).

3.7.1.2 Growth and age effects. Controls for seasonal and spatial effects on metal accumulation will likely also control for growth, although further precautions may be taken to reduce natural variation in metal uptake. By collecting molluscs within a narrow size-age range (e.g. individuals 80-100 mm in length and 7-10 yr.) the investigator can be relatively confident that the individuals possess similar growth rates. Size in bivalves is usually determined by length (Hinch and Stephenson, 1987) and age is determined by

counting external annuli in unionids (Metcalfe-Smith and Green, 1992). Determining the age of marine mussels, gastropods and Sphaeriid sp. is difficult if not impossible; length is therefore used to obtain individuals with similar growth rates. Metcalfe-Smith and Green (1992) recommend that only bivalve species that can be easily aged be used in biomonitoring programs. Size alone in not often a good indicator of growth since bivalves that are 80-100 mm in length may be 4 or 15 years old because they possess fast or slow growth rates, respectively.

3.7.1.2 Choosing Species and Sex. Bivalves and gastropods have many characteristics that make them suitable for biomonitoring studies. Snails are common in freshwater, but they lack supporting scientific information on metal accumulation which makes them less desirable as sentinel organisms. Further, snails are small and extracting them from their shells for tissue analysis may be problematic in routine field assessments. For these reasons gastropods are not recommended for use as sentinel organisms at this time.

Of the bivalve species, the major groups include fingernail clams (Sphaeriidae), long-lived Unionids and introduced *Dreissena polymorpha*. Sphaeriid clams have not been widely used in biomonitoring programs because they are small and their soft tissues are difficult to remove. *Dreissena polymorpha* is limited in distribution to the Great Lakes and there is strong motivation to prevent any further invasion of other parts of the country, ruling out transplant studies. Since there are no other similar species, *Dreissena* is not recommended for a nation-wide biomonitoring program, but may be suitable for programs specific to the Great Lakes. *Dreissena* is currently being used in biomonitoring throughout Europe (Kraak et al. 1991; Mersch et al. 1996).

Unionid species offer the greatest number of advantages due to their ubiquitous distributions and large size. Some Unionid species are more widespread than others (e.g. *Pyganodon grandis*, *Elliptio complanata*), making them suitable for a nation-wide program. However, in many cases the choice of species will be site-specific depending on availability and abundance. Unionids are not recommended for collection in the United States National Water-Quality Assessment (NAWQA) Program because many species are considered endangered (Crawford and Luoma, 1993). Although there are a

number of species that have not declined in abundance and would be suitable as biomonitors, organizers of NAWQA Program are concerned that endangered species may be collected accidentally along with the healthy species. The NAWQA Program instead utilizes the introduced asiatic clam *Corbicula fluminea* which is spreading throughout waterways in the U.S. Before a unionid species is chosen for any biomonitoring program its conservation status must be carefully considered, for the present time and the duration of the monitoring program.

The marine mussel *Mytilus edulis* has been chosen for use in the Mussel Watch (O'Connor, 1992) and is recommended for marine monitoring programs in Canada.

Several authors have found that metal accumulation varies among unionid species in a metal-specific manner (Metcalfe-Smith, 1994; Lobel et al. 1990). Inter-specific differences may exist in absolute metal concentrations if correlations are in the same direction, species could be used interchangeably after conversion by means of regression equations. For example, *Elliptio complanata* and *Lampsilis radiata radiata* could be used interchangeably to monitor site-to-site trends in the bioavailability of Cd and Pb (no inter-specific difference exist), and after conversion by means of regression equations to monitor Al, Cr, Cu, Fe, Hg and Ni (Metcalfe-Smith, 1994). There are no easy answers in this case. Samples will have to be taken to determine if and for what metals inter-specific differences exist at a particular site before concentrations can be compared between species.

There are differences in metal accumulation between the sexes of unionids, but they are less pronounced that inter-specific differences. Determining the sex of unionids from morphological attributes is only possible for one species (*Lampsilis radiata*), making sex-specific collections impossible. Therefore, it is recommended that collections be taken prior to fertilization when sex-specific metal differences are minimal and/or enough samples be collected so brooding females can be rejected from metal analyses.

3.7.2 Transplanting and caging molluscs

Transplanted molluscs can be useful tools in biomonitoring programs provided the method of caging does not cause undue stress to the animals and affect normal metal uptake. Many studies have documented the use of transplanted and caged bivalves (Couillard et al. 1993 and 1995a,b; Salazar et al. 1996). The following are some of the approaches and problems associated with transplanting and caging molluscs.

3.7.2.1 Methods of caging. The method of caging should take into consideration a number of factors:

1. Cages in metal accumulation studies should not be made of metals or products that are degradable and release metals or enhance metal adsorption. Plastic is often the material of choice. Colored plastic should be used with caution since many dyes contain Cd and Pb.
2. Cages should allow for good circulation of water and particulate material. Mesh sizes 4 mm and larger appear to a acceptable. Light penetration should also be considered in cage development. Some bivalves show diurnal patterns of valve-openness corresponding with light that may affect metal uptake (McCorkle et al. 1979).
3. Whether or not the cage allows the bivalves contact with the sediment depends on the species and the study objectives. Bivalve behavior will be disrupted less if the cages are in contact with the sediment, particularly for those species (i.e. unionids) normally found in contact with the sediment.
4. Cages or compartments should prove enough space so as to not cause over-crowding. Natural densities of bivalves are species specific and efforts should be made to ensure these densities are not exceeded.
5. Cages that completely enclose the bivalves tend to reduce predation, but they can also reduce water flow past the bivalves as well as light intensity.

The reader is referred to studies by Couillard et al. (1993 and 1995a,b), Salazar et al. (1996) and Malley et al. (1996) for three different methods of caging freshwater bivalves for metal studies.

3.7.2.2 Effect of transplanting, marking and caging on molluscs. Little is known about the effects of transporting and caging molluscs. It is not unlikely that moving to a new environment results in stress, but does it effect survival and metal accumulation; are there consequences for metal accumulation studies? Preliminary studies using the freshwater mussel *Pyganodon grandis* caged in the field and held in the laboratory revealed that blood ion composition (physiological indicator of stress, Malley et al. 1988) of caged mussels was not significantly different from non-caged mussels from the same lake (D.F. Malley, unpublished data). On the other hand, mussels transported and held in the laboratory showed significant differences in blood ion composition from the same non-caged mussels. The changes in blood ion composition may be a reflection of the physical transport process to the laboratory rather than the actual holding the laboratory. If transportation causes stress then caging studies that require mussels to be physically move from one system to another will likely result in stressed animals. Further studies are needed to confirm the causes of stress in mussels and to determine the consequences for metal uptake in bivalves.

Information on the effects of transplanting and caging bivalves is sparse. Results are inconsistent as well as species-, metal- and site- specific. A common result of caging is the loss of soft-tissue weight throughout caging periods ranging from 80-400 days (Couillard et al. 1995a; Malley et al. 1996; Salazar et al. 1996). Metal concentrations will have to be adjusted for weight losses (i.e. expressed as body burdens = concentration ($\mu g\ g^{-1}$) x organism (or tissue) weight (g)). Mortality is also a common effect of many caging studies. Survival rates of up to 85-90 % are possible, but in certain situations (low oxygen, high temperature, toxicity) high mortality results. Sample sizes should take into consideration potential losses and cages should be checked regularly for mortalities. Losses of more than 2 mussels often indicate a larger problem and if they are detected early the cages could be moved to an alternate site.

Marking transplanted or caged bivalves is often done so that the growth of individual specimens can be tracked throughout the exposure period. The results of field studies by Couillard et al. (1995) indicate that marking techniques can effect bivalve mortality. They found that scratching a number into the shells of the bivalves with a nail resulted in the death of several animals due to accidental puncture of the shell. However, subsequent experiments where bivalves were marked with a glued-on tag mortality rates improved (Couillard et al. 1995a). Careful consideration should be given to the mollusc marking technique used in field studies to ensure specimens are not lost.

3.7.2.2 Collecting and rearing. The collecting process should not injure or contaminate the bivalves. Since freshwater bivalves are usually found in depths less than 6 m, collections can be done relatively easily using SCUBA or in shallow instances, snorkeling. Marine bivalves can also be collected using SCUBA. Species can be identified in the field and require only a modest understanding of bivalve taxonomy.

Aquaculture has improved techniques for rearing marine mussels, but rearing techniques for freshwater bivalves are limited and labor intensive. Most unionids require a specific fish host for larval development, a stage that is often difficult to achieve in the laboratory. Rearing of unionid populations would be advantages in that bivalves used in transplant studies would possess the same genetic make-up and their "source" environmental conditions could be controlled. Further research into rearing of freshwater bivalves in the field is recommended, particularly in light of recent information on the declining status of unionids in North America.

3.8 Recommendations

Compared to fish or plants, molluscs offer several advantages as biomonitoring organisms, meeting most of the criteria outlined in section 3.2.2. In the U.S., the bivalve *Corbicula fluminea* was recommended as the top priority for sample collections in the NAWQA Program, over fish and plants (Crawford and Luoma, 1993). Therefore, molluscs, bivalves in particular, are likely candidates for monitoring programs for the

mining industry. Before describing how they might be used it would be useful to review the additional factors outlined in section 3.3.2 that are specifically relevant to biomonitoring tools for the mining industry:

1. *Do molluscs occur naturally in mining impacted areas, and if not can they be artificially introduced in cages?* Yes, bivalve species occur in mining impacted areas (e.g. Rouyn-Noranda) and they have been successfully transplanted and caged in mining areas.
2. *Are there differences in metal accumulation patterns between naturally occurring indigenous populations and transplanted molluscs?* Yes, in some cases the patterns of uptake are different and thus, metal accumulation in indigenous populations and transplanted populations cannot be considered the same until proven otherwise.
3. *Are there physiological constraints to using molluscs to monitor areas receiving mining discharge, e.g. pH, [Ca]?* Yes, bivalves have absolute lower pH limits of 4.7 and [Ca] limits of 2 mg L^{-1} and are restricted to waters above these lower limits. Bivalves would not be suitable to monitoring areas directly receiving acid-mine discharge.
4. *Are molluscs useful for detecting short-term pollution events (episodic events) or long-term trends in the receiving environment?* Bivalves could be used to monitor both short- and long-term trends. Fast-growing, short lived Sphaeriid species or *Dreissena polymorpha* and transplanted unionids could be used to detect short-term trends (e.g. 1 to 6 months) and long-term trends could be monitored by indigenous long-lived unionid species (e.g. years).
5. *Can tissue metal concentrations be related to different sources of metal in the aquatic environment?* There is potential for bivalves to detect different sources of metals (e.g. water/particulate, point-sources), but this requires extensive sampling of the external conditions and rigorous experimental design.

Based on the answers, the following are recommendations of the use of molluscs as biomonitoring tools for the mining industry. The recommendations will be provided based on a series of questions posed by the AETE Program committee.

1. *Is the tool an indicator of exposure or response?* Molluscs are indicators of exposure to metals such as Cd, Cu, Zn, Pb, Ni, Hg, As, Cr, and Ag. Metal-induced effects in molluscs such as changes in growth, or MT concentration are not well established. These responses could be measured as part of the monitoring program, but the results should be used with caution until their role has been validated in field studies using mining contaminants.
2. *What information does the tool provide?* Bivalve molluscs could be used to:
   a) confirm changes in biologically-relevant metal concentrations in the natural environment resulting from mining activities.
   b) monitor long-term spatial and temporal trends in biologically-relevant metal concentrations by increases and decreases in tissue concentrations of indigenous or transplanted populations.
   c) determine the effectiveness of remedial measures through the use of transplanted and indigenous molluscs.
3. *Does the tool require supporting information for proper interpretation?* Proper interpretation of bivalve tissue metal concentration requires rigorous collection of external physical and chemical variables including metal concentrations in sediment (metals are partitioned into bioavailable fractions, e.g. Fe-ox bound metals, free-ion concentrations), water chemistry (i.e. $[Ca^{2+}]$, pH, DOC etc.), temperature and food availability and quality. These additional variables are critical in order to derive useful relationships between metal concentrations in the environment and tissue metal concentrations.
4. *Level of action for tool?* Molluscs may be used in the first steps of a monitoring program in order to assess the extent of metal contamination in the aquatic environment. In more detailed information stages, molluscs may be used to

investigate specific sources of bioavailable metals, improvements to waste-water treatment and effectiveness of remedial actions.

5. *Best use of the tool in the overall monitoring strategy of the mining industry?* For comprehensive biomonitoring, molluscs can be used in conjunction with several other organisms (e.g. invertebrates, fish, plants) to monitor metals and their effects in the aquatic environment. In this way, information on the "biological consequences" of mining operations can be obtained, that is not available from monitoring sediment and water only.

CHAPTER 4. ACCUMULATION OF CADMIUM BY A FRESHWATER MUSSEL (*PYGANODON GRANDIS*) IS REDUCED IN THE PRESENCE OF Cu, Zn, Pb AND Ni

4.1 Abstract

The effect of a mixture of metals (Cu, Zn, Pb and Ni) on Cd availability and uptake by freshwater mussels (*Pyganodon grandis*) was examined in a limnocorral experiment in a Precambrian Shield lake during the summer of 1992. Cadmium was added alone to treatment 1 and with the metal mixture (at increasing concentrations) to treatments 2-4 to raise background sediment Cd concentrations by 7 times. Copper, Zn, Pb and Ni were added to treatments 2, 3 and 4 to raise sediment concentrations by 3, 4 and 7 times, respectively. After receiving metal additions over a period of eighteen days, mussels were introduced into the limnocorrals at t=0 (13 July 1992). Water metal concentrations at t=0 ranged from 4 - 14 $\mu g\ L^{-1}$ Cd, 12 -31 $\mu g\ L^{-1}$ Cu, 4 - 414 $\mu g\ L^{-1}$ Zn, 30 - 87 $\mu g\ L^{-1}$ Pb, 18 - 57 $\mu g\ L^{-1}$ Ni. The limnocorrals were sampled three times at t=0, 40 and 80 days. Treatments with the metal mixture had longer residence times for Cd in the water column than the treatment with Cd alone. Cadmium accumulation in mussels was significantly reduced in treatments with the highest concentration of the metal mixture compared to treatments with the lowest concentration of the metal mixture or with Cd alone. This trend was consistent for the individual tissues; gill, mantle, foot and kidney. Metallothionein (MT) levels were highest in the kidney and tended to decrease among treatments with increasing metal addition. The effect of competition on the partitioning of Cd in water column appeared to be less important than competition at binding sites on the mussel in determining Cd uptake by the mussels.

4.2. Introduction

In the environment, contaminants are generally present as mixtures. For example, toxic heavy metals from non-ferrous metal mining, smelting and refining processes are released together into aquatic ecosystems through atmospheric deposition (Nriagu 1990; Scheider et al. 1981; Jeffries and Snyder, 1981), surface run-off, and milling effluents (AQUAMIN, 1996; Kelly, 1988). The resulting mixture of heavy metals in the receiving waters may interact and mutually influence metal toxicity to aquatic organisms.

Despite the fact that metals are present in the environment as mixtures, water and sediment quality criteria continue to be developed for contaminants singly (Jaagumagi, 1992; CCREM, 1987). In order to protect aquatic life from the damaging effects of heavy metals, the combined effects of individual metals should be considered. Nevertheless, metal mixtures are complex and many published results are contradictory or ambiguous making it difficult to develop a pragmatic approach for dealing with the mixtures (Kraak et al. 1994; Keller and Zam, 1991; EIFAC, 1987; Spehar and Fiandt, 1986; Voyer and Heltshe, 1984).

Most of the published studies on metal mixtures consist of controlled laboratory experiments using filtered and sterilized test water and no sediment. This is done to control physical and geochemical factors that might affect metal bioavailability in the test system. Removal of these factors normally present in the aquatic medium means that the role of metal interactions in determining individual metal availability for uptake by organisms is not considered. Thus, the observed interactions among metals and their effects on metal uptake or toxicity are unlikely to be representative of natural systems.

Metals introduced to surface waters are eventually lost to and accumulate in the sediment. This transport to the sediment can occur by adsorption to settling particles in the water column or to particles at the sediment-water interface (Santschi et al. 1986; Campbell and Tessier, 1996; Luoma, 1983; Hart, 1982). Metals remaining suspended in the water column can be accumulated by phytoplankton, zooplankton, filter feeding macro-invertebrates, and fish (Campbell and Tessier, 1996; Luoma, 1983; Morel, 1983). Therefore, factors that limit the removal of metals from the water column and sequestration in bottom sediments are of interest. Field studies that provide insight into

the behavior of metal mixtures and their effect on the partitioning of metals in natural systems are needed because laboratory studies alone are often inadequate.

Cadmium, a priority substance under the Canadian Environmental Protection Act (CEPA), was chosen for study here because it is highly toxic to aquatic organisms and is commonly released with the metals Cu, Zn, Pb and Ni during mining, smelting and other industrial processes (Nriagu, 1990; Nriagu, 1980). Cadmium is the subject of a whole lake addition experiment at the Experimental Lakes Area (ELA) in northwestern Ontario to test the fate and distribution of low level Cd, and its effects on aquatic biota (Malley 1996). In this whole lake experiment, Cd and its radiotracer ^{109}Cd were added to Lake 382 during the ice-free seasons from 1987 to 1992 to bring the epilimnetic Cd concentration near the 1979 Canadian Water Quality Guideline of 0.2 $\mu g\ L^{-1}$. Results from the whole lake experiment are presented by Stephenson et al. (1996), Lawrence et al. (1996), and Findlay et al. (1996). The present experiment provides information on metal mixtures that complements data from the whole lake experiment on Cd alone.

The influence of a metal mixture (Cu, Zn, Pb and Ni) at three concentration levels on the partitioning of Cd in the aquatic environment and its accumulation by the freshwater floater mussel, *Pyganodon grandis* (formerly *Anodonta grandis grandis*) was examined in a limnocorral experiment in a lake at the ELA. The highest concentrations used in the experiment were set to mimic representative sediment concentrations found in lakes near base-metal smelters (Harrison and Klaverkamp, 1990). The removal rate of Cd added to water column and the partitioning of Cd among water, suspended particles and sediment are described. *Pyganodon grandis* has previously been used to assess the bioavailability of metals in contaminated environments (Couillard et al. 1993 and 1995; Tessier et al. 1993; Stewart and Malley, 1994; Malley et al. 1996). Concentrations of Cd and the other metals in the whole-body and soft-tissues of *P. grandis* are described. The effects of the metal mixture at the sub-cellular level were also monitored by the development of metal-binding protein metallothionein (MT).

4.3 Methods

Experimental Site

The Experimental Lakes Area (ELA) is located 52 km southeast of Kenora, Ontario on the southwestern part of the Precambrian Shield (Brunskill and Schindler, 1971; Malley and Mills 1992). The ELA comprises some of the most oligotrophic lakes in the world. They are representative of small lakes especially prone to acidification and contamination from metals and organic pollutants (Schindler, 1988). Apart from small scale logging in previous years, limited fishing and hunting, past occurrence of forest fires, and regional LRTAP deposition, the ELA remains pristine.

The site of the limnocorral experiment was a shallow, protected bay in Roddy Lake (93 43' W, 49 41' N) (Fig. 4.1). Water chemistry for Roddy Lake is shown in Table 4.1. The experimental site consisted of oxic littoral sediment composed of sand and gravel (1-2 % loss on ignition (LOI)) down to a depth of 6 - 8 cm, below which there was clay. There was a slight gradient of increasing sedimentary organic matter (OM) (~ 10% difference) from north to south along the transect where the limnocorrals were situated. The bay contained isoetid macrophytes including *Eriocaulon septangulare* and *Juncus sp.*, and a few resident mussels, *Pyganodon grandis*.

Experimental Design

The experiment consisted of four duplicated treatments (n=2) for a total of 8 limnocorrals (Fig. 4.1). Treatments 1-4 received Cd additions targeted to raise sediment Cd concentrations by seven times above background(Table 4.2). Treatments 2, 3 and 4 also received a mixture of Cu, Zn, Pb and Ni to raise sediment Cu, Zn, Pb and Ni concentrations by three, four or seven times above background, respectively (Table 4.2). To compensate for the gradient in OM that could affect metal bioavailability and therefore confound the results, each treatment replicate was randomly assigned within a southern block (A - lower organic matter) and a northern block (B - higher organic matter) (Fig. 4.1). This experimental design reduced the chance that statistical differences among treatments were the result of the gradient in OM rather than a treatment effect (Hurlbert 1984). No statistically significant differences between the two blocks were found for any of the variables tested ($P>0.05$).

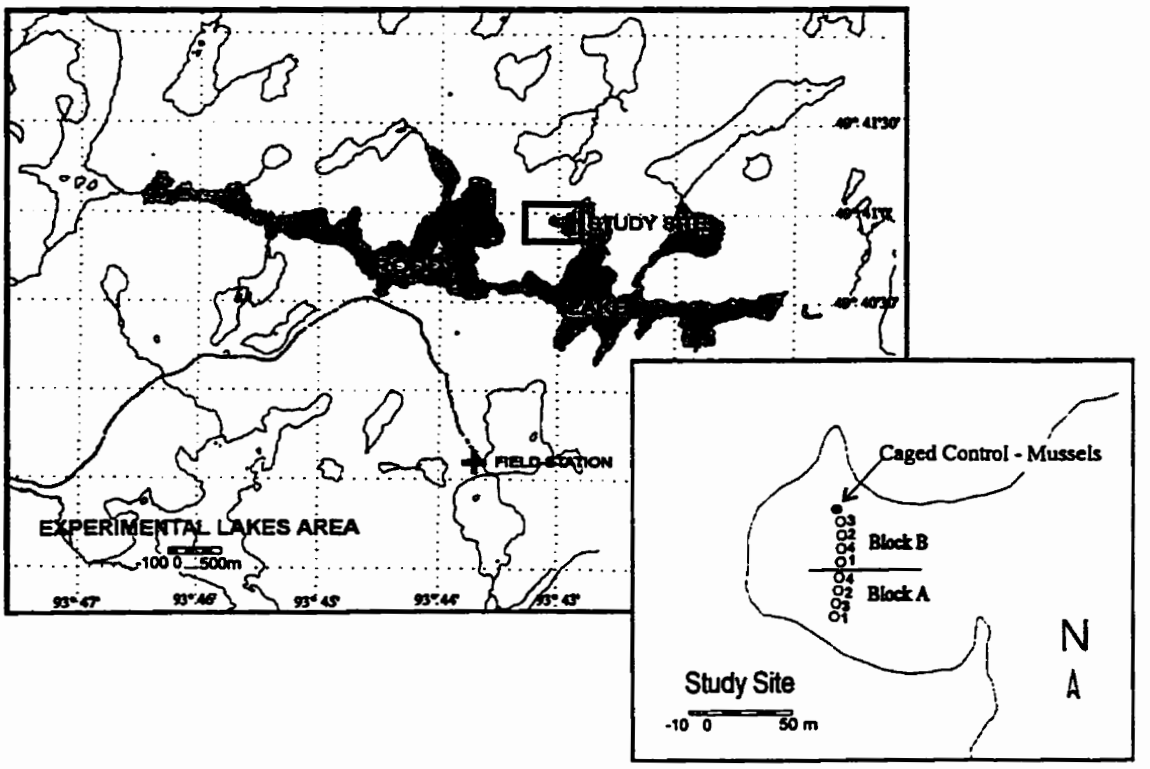


Figure 4.1. Location of study site in Roddy Lake at the Experimental Lakes Area. Location of limnocorrals and treatment assignment between blocks is shown.

Table 4.1. Surface water chemistry of Roddy Lake and Lake 104. Values for Roddy Lake are means (±SD) of 6 samples from 8 June to 15 October 1992. Values for Lake 104 are based on a single sample taken on 15 September 1992.

Parameter	Roddy Lake	Lake 104
NH_4-N, $\mu g\ L^{-1}$	9.7 ± 4.0	9
DIC, $\mu mol\ L^{-1}$	143 ± 8	120
DOC, $\mu mol\ L^{-1}$	413 ± 23	940
Na^+, $mg\ L^{-1}$	1.00 ± 0.05	0.98
K^+, $mg\ L^{-1}$	0.43 ± 0.07	0.44
Ca^{2+}, $mg\ L^{-1}$	2.41 ± 0.12	2.53
Mg^{2+}, $mg\ L^{-1}$	0.68 ± 0.02	0.68
Fe, $mg\ L^{-1}$	0.02 ± 0.01	0.14
Mn, $mg\ L^{-1}$	0.01 ± 0.0	0.01
Cl^-, $mg\ L^{-1}$	0.37 ± 0.02	0.24
SO_4, $mg\ L^{-1}$	3.23 ± 0.09	1.64
Alkalinity, $\mu eq\ L^{-1}$	134 ± 4.7	123
pH	6.74 - 7.27[a]	6.59
O_2, $mg\ L^{-1}$	9.15 ± 0.41	8.7

[a] Range

Table 4.2. Experimental design. Values are the factor by which background sediment metal concentrations were targeted to be increased. Each treatment was represented by 2 limnocorrals.

	Treatment			
Metal	1	2	3	4
Cd	7X	7X	7X	7X
Cu	1X	3X	4X	7X
Zn	1X	3X	4X	7X
Pb	1X	3X	4X	7X
Ni	1X	3X	4X	7X

Mussels were introduced into the limnocorrals at two times, day 0 when metal levels were high and day 40 when metal levels were low to compare responses to two exposure levels. Additional mussels were caged outside the limnocorrals to serve as transplant controls. The control cage was open to the sediment and composed of plastic mesh (1 cm diameter, 250 cm high borders), allowing unrestricted exposure of the mussels to lake water. Mussels were free to move within the limnocorrals and the cage. The aquatic macrophyte *Eriocaulon septangulare*, was also introduced into the limnocorrals on day 0. The accumulation of metals in *E. septangulare* introduced into the limnocorrals will be described elsewhere.

The limnocorrals were 2 m diameter cylindrical tubes approximately 1.5 m deep, composed of reinforced woven translucent plastic (Curry Industries, Winnipeg, MB). At the lake surface they were supported by Styrofoam floats, each in a reinforced vinyl pocket closed with plastic ties and supported by an external aluminum frame. The bottom of the limnocorrals ended in skirts that extended outward onto the sediment surface and were secured on the sediment with sandbags placed by snorkel divers.

Metals were added to limnocorrals as salts ($CdCl_2 \cdot 2½ H_2O$, $CuCl_2 \cdot 2 H_2O$, $ZnCl_2$, $Pb(NO_3)_2$ and $NiCl_2 \cdot 6 H_2O$; Fisher Scientific, ACS grade) dissolved in 1 L distilled

deionized water in five separate additions on 5, 16, 17, 19 and 22 June 1992. The exposure experiment began on 13 July 1992 (day 0), three weeks after the final metal addition.

Collection of mussels

The source of the mussels *Pyganodon grandis* (Say) Hoeh, introduced into the limnocorrals was Lake 104 (93 50' W, 49 41' N). Lake 104 is a brown water lake with higher DOC and slightly lower pH than Roddy (Table 4.1). Transplant of the mussels to Roddy Lake did not expose them to an appreciable change of water quality. On day 0 and day 40 (27 August), 180 and 25 mussels, respectively, of ~9 yr of age and 10 ± 0.8 cm in length (mean ± SD) were collected by snorkel divers and transported in coolers to Roddy Lake. These mussels were introduced into 8 limnocorrals and into the control cage (Fig. 4.1).

Water and Sediment Sampling

To monitor the loss of metals from the water column, water samples were collected from Roddy Lake and limnocorrals in acid-washed polyethylene Nalgene® bottles every 2 days from 5 June 1992 to 28 July 1992, and once a week thereafter until 15 October. Total water samples (unfiltered) were acidified (0.5 % HNO_3, Baker Analyzed - For Trace Metal Analysis) immediately upon returning to the laboratory. The partitioning of metals in the limnocorral water column on days 0, 40 and 80 was determined in water samples drawn from 1m depth using a peristaltic pump. The 1-m water samples were filtered through 10μm nylon mesh netting into a Nalgene® carboy to avoid clogging of the 1 μm filter and the >10 μm particulates were discarded. At the field laboratory, the remaining water (~2.5 L) was filtered through a 1μm mesh 142 mm diameter polycarbonate membrane filter (Poretics Corp., Mississauga, Ont.) using a vacuum pump. Samples of the <1μm filtrate and a laboratory blank of 1μm filtered distilled deionized water were taken. The 1 μm mesh was chosen rather than the commonly used 0.45 μm because the 1 μm cut-off is closer to the particle size retained by filter-feeding unionid bivalves. Analysis of stomach contents suggests that the smallest particle size retained by *Pyganodon* species is 8 μm diameter (Tankersley and Dimmock,

1993). Furthermore, the mesh size of 1 μm is used to operationally define suspended and dissolved fractions by the ELA Chemical Laboratory. Use of the 1 μm mesh allowed for direct comparisons between metal and ELA water chemistry data.

Additional water samples were taken from Roddy Lake and limnocorrals 1A, 2B, 3A and 4B once a month in June, July, August and September for analysis of major ions (Na, K, Ca, Mg, Fe, Mn), pH, alkalinity, suspended C, N and P, dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC), nitrate, nitrite and ammonia, total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), soluble reactive Si, sulfate (SO_4^-) and organic acids, conductivity, and oxygen. Chemical analyses were performed using methods from Stainton et al. (1977) with the following modifications. Dissolved organic carbon samples were analyzed by a heated persulfate digestion followed by total carbon analysis (Total Carbon Analyzer Model 700, O I Corp.). Dissolved inorganic carbon was measured using infrared detection of CO_2. Water samples for Cl^- were taken weekly from the limnocorrals and Roddy Lake beginning 15 July 1992 until the end of the experiment.

Chloride samples were used to monitor the limnocorrals for leakage. Because most metals were added as Cl^-, Cl^- salt levels should have remained elevated above the ambient lake water. From the time of final metal addition to day 80, Cl^- levels in the treatments decreased by approximately 2-12 %, after adjusting for changes in limnocorral water volume due to precipitation and evaporation. This amount of leakage is less than that observed in other ELA limnocorral experiments (Santschi et al. 1986).

To determine if target levels were achieved in limnocorral sediments, metal concentrations were measured in sediments on day 0, 40 and 80. Samples were collected in triplicate with a 5-cm internal diameter plexiglass coring tube. The top 2 cm of the core was placed in an acid-washed centrifuge tube and centrifuged at 4,000 rpm for 30 min to remove porewater. Sediments were then frozen at -30 °C until analyzed for metals.

Sampling and processing of mussels

On day 80, mussels were collected from the limnocorrals by SCUBA divers, placed in clean polypropylene bags and transported to the field laboratory for processing.

Mussels introduced into the limnocorrals on day 0 and removed on day 80 (15 October) were designated 0-80 d and mussels introduced on day 40 and removed on day 80 were designated 40-80 d. Mussels were also collected from Lake 104 on day 0, 40 and 80 for background metal determinations. Mussels were maintained without water at 7.5 ● 0.5°C (lake temperature on day 80) until they were processed within 12 hrs. No specific attempt was made to clear gut contents, since previous metal uptake experiments carried out at the ELA showed that gut clearing had little effect on whole body or tissue contents of Cd (Malley et al. 1989).

Of the 205 mussels introduced into the limnocorrals and cage, 6 died (3 from limnocorral 4B) and 30 mussels were not be recovered until the following spring. The spring mussels were not included in the following analyses. Mussels were blotted dry, and their shell length, height, thickness and live weights were measured. Length, height, depth and live weight of mussels did not differ significantly ($P>0.05$) among groups in the limnocorrals or cage. Mussels were removed from the shells and whole bodies were frozen in whirl-paks or they were dissected into mantle, gill, foot, kidney and remainder of body or viscera. Mussel shells and bodies were freeze-dried (Lab Con Co. Freeze Dry 5, Fisher Scientific Co., Winnipeg, MB) and condition of the mussels was determined by the ratio of freeze-dried (fd) body weight:freeze-dried shell weight after Davenport and Chen (1987):

$$\text{Condition} = \frac{\text{fd body wt (g)}}{\text{fd shell wt (g)}}$$

Mussels for analysis of metallothionein (MT) were dissected into tissues, that were placed into whirl-pak bags and frozen immediately on dry ice. Samples for MT were not stored under N_2 (as described in Couillard et al. 1995), but were kept frozen in a –80°C freezer until they were analyzed within 4 months.

With few exceptions, mussels bearing glochidia were not included in the statistical analyses. Metal concentrations were determined for the whole bodies of 0-80 d and 40-80 d mussels from each limnocorral and cage (5 animals per limnocorral or cage). Tissue metal concentrations were also determined for 0-80 d mussels from limnocorrals 1A, 2B, 3A, and 4B (4-5 animals) and the cage (2 animals). Background metal concentrations were determined for the whole bodies of Lake 104 mussels on day 0, 40

and 80 (minimum of 6 animals) and in individual tissues on day 80 (3 animals). Metallothionein concentrations were determined for individual tissues of 0-80 d mussels from limnocorrals 1A, 2B, 3A and 4B (5 animals) and 0-80 d mussels from the cage (3 animals). Background metallothionein concentrations were determined for individual tissues of Lake 104 mussels on day 80 (6 animals).

Analysis of water, sediment and tissues

Total water samples and <1 μm filtrate were analyzed for metals by graphite furnace atomic absorption spectrophotometry (GFAAS) and flame atomic absorption spectrophotometry (FAAS) on a Varian GTA-95 (Varian Instruments, Georgetown, Ontario). The 1 μm filtered blanks (n=3) were all below the analytical detection limit (DL) for each metal (Cd <0.02 $\mu g \cdot L^{-1}$, Cu <0.5 $\mu g \cdot L^{-1}$, Zn <0.5 $\mu g \cdot L^{-1}$, Pb <0.3 $\mu g \cdot L^{-1}$, Ni <0.5 $\mu g \cdot L^{-1}$). Precautions were taken to prevent contamination by soaking all glassware in conc. HNO_3 (Reagent grade) and rinsing it 3 times in distilled deionized water.

Sediments were freeze-dried, digested by Aqua Regia (4 HCl: 1 HNO_3) and the extracts were analyzed for metals using FAAS and GFAAS. Duplicate samples (~100 mg) of NRCC reference sediments, MESS-1 and PACS-1, were analyzed with almost every sample run. Mean (±SD, n=4-6) metal concentrations obtained for the reference materials were within the range specified for certified values (in parentheses): Cd - 2.47 ● 0.2 (PACS-1: 2.38 ● 0.2); Cu - 22.2 ± 1.8 (MESS-1: 25.1 ± 3.8); Zn - 161 ± 6.4 (MESS-1: 191 ± 17); Pb - 29.7 ± 4.4 (MESS-1: 34.0 ± 6.1); Ni - 42.0 ± 3.6 (PACS-1: 44.1 ± 2.0). Variability (coefficient of variation, CV) among triplicate cores from individual limnocorrals for the different metals were: Cd ~15%, Cu ~20%, Zn ~15%, Pb ~21%, and Ni ~21%.

Freeze-dried mussel bodies and tissues were ground in a coffee grinder for at least 15 seconds. Tissues were then digested using concentrated HNO_3 and an H_2O_2 oxidation step according to Malley et al. (1989). Metal concentrations were measured with Flame Atomic Absorption Spectrophotometry and GFAAS using a Varian GTA-95 or polarized Zeeman Z-8200 with Zeeman background correction (Hitachi Scientific Instruments, Canada). Duplicate samples of National Research Council (NRC) reference materials Dolt-2 and Dorm-1 (~100mg) were analyzed with every set of mussel samples.

Measured Cd, Cu and Zn concentrations in reference materials varied little over time (CV 3-11%, n=7-16) and were within the specified ranges for each metal. Lead and Ni concentrations were more variable (CV 18-45%, n=10), but their means were within the certified ranges. Average CVs for whole mussel metal concentrations calculated for mussels collected from each limnocorral were: Cd 18%, Cu 21%, Zn 16%, Pb 28% and Ni 27%. The variation in metal contents of individual tissues were generally 1.5 to 2 times higher than the above CVs. The above CVs for whole bodies were similar to those reported in other field studies with *P. grandis* (Couillard et al. 1993).

Metallothionein was determined by a ^{203}Hg-displacement method (Klaverkamp et al. 1991). ^{203}Hg activities in the samples were expressed as nanomoles of metal binding sites per gram dry weight (based on measured Hg binding capacities).

All sediment concentrations and whole body and tissue metal levels were expressed on a dry weight basis unless otherwise specified.

Calculations and Statistics

The loss rate for Cd from the water column in the different treatments was calculated as a half-life, in days:

$$b = \frac{\ln C_o - \ln C_t}{t}$$

$$t_{½} = \frac{\ln 2}{b}$$

where b is the loss rate coefficient, C_o is the total water metal concentration on 24 June 1992, 1 day after the final metal addition to the limnocorrals, C_t is the total water metal concentration at the end of the experiment on 15 October 1992 and t is the number of days between C_o and C_t (113 d).

Apparent partitioning coefficients, K_d^a, for Cd on particulate material and sediment were calculated for days 0, 40 and 80 according to Wood et al. (1995):

$$\text{particulate } K_d^a = \frac{C_p}{C_f C_{ss}}$$

$$\text{sediment } K_d^a = \frac{C_s}{C_f C_{ss}}$$

where c_p was the concentration of Cd adsorbed to >1 μm particles (moles kg^{-1}) which was equal to the Cd concentration in the total water sample ([Cd(total)]) minus the Cd concentration in the 1 μm filtrate ([Cd(filtrate)]), c_s was the concentration of Cd in sediments (mole kg^{-1} dry sediment), c_f was [Cd(filtrate)] (mole kg^{-1}) and c_{ss} was the concentration of suspended solids (mg L^{-1}). Partitioning coefficients were termed "apparent" since the system had not reached equilibrium.

The Cd body burden or "content" for mussel whole bodies and tissues were calculated, where:

Cd body burden (μg) = Cd conc. (μg g^{-1}) x tissue weight (g).

Concentrations in the whole body, [MT(body)] were calculated as:

$$[\text{MT(body)}] = \frac{\sum [\text{MT(tissue)}]_i W_i}{\sum W_i}$$

where $[\text{MT(tissue)}]_i$ is the metallothionein concentration and W_i the dry weight of the *i*th tissue.

Data were tested for normality using the Shapiro-Wilk statistic, a ratio between the best estimator of the variance and the corrected sum of squares estimator of the variance, and log transformed when required. Statistically significant differences in mussel size and metal concentrations in water, particulate material and mussels among treatments were determined using SAS version 6.08 ANOVA, randomized blocks design (SAS Institute Inc., 1989). Differences in mussel condition and background tissue metal concentrations among Lake 104, the cage and treatments were also determined using ANOVA procedure. Correlations between MT concentration and metal concentration in the body parts were determined using SAS version 6.08 correlation analysis, with Pearson and Spearman correlation coefficients.

4.4 Results

Metal concentrations in sediments

Cadmium concentrations in limnocorral sediments reached target concentrations by day 0 in treatment 1, by day 40 in treatments 2 and 3, but not until day 80 in treatment 4. Target concentrations of the other metals were reached in the sediments by 40 or 80 days (Table 4.3). Nickel concentrations in all treatments were slightly below target levels

and Pb concentrations in treatment 3 and 4 limnocorrals exceeded target levels. Surprisingly, all of the metals were elevated in treatment 2 sediments (only in limnocorral 2A) on day 40 relative to day 80.

Loss of Cd and other metals from the water column

Cadmium remained in the water column longer in treatments with the metal mixture than with Cd alone (Fig. 4.2, Table 4.4). The half-life for Cd in treatment 1 was 18 ± 0.1 d (mean ± SE) compared to 24 ± 1.3 d, 29 ± 0.4 d and 28 ± 3.6 d for treatments 2,3 and 4, respectively. Water column [Cd(total)] in treatment 1 was significantly less than in treatments with the metal mixture on day 0 ($F_{3,3}$=19.97, P=0.02) and 40 ($F_{3,3}$=40.54, P=0.006), but not day 80 (Table 4.4). The [Cd(total)] was consistently higher in treatment 4 vs. treatment 3 and treatment 3 vs. treatment 2, but the differences were not significant on days 40 and 80. As expected, Cu, Zn, Pb and Ni concentrations in the water column of treatments 2-4 increased with increasing addition of the mixture and declined with time over the course of the experiment (Table 4.4). Copper, Zn, Pb and Ni concentrations in treatment 1 remained low or below detection limits.

The trends in the Cd concentration in the <1 μm filtrate ([Cd(filtrate)]) were similar to those for [Cd(total)] on each sample day. Values for [Cd(filtrate)] were significantly higher in treatments with the metal mixture compared to that in treatment 1 on days 0 ($F_{3,3}$=32, P<0.01), 40 ($F_{3,3}$=13, P<0.05) and 80 ($F_{3,3}$=13, P<0.05) (Fig. 4.3).

The apparent partitioning coefficients for Cd on particles (K_d^a) were considerably more variable than those for the sediment (K_d^s) (Fig. 4.4). The particulate K_d^a for treatment 1 progressively decreased from day 0 to day 80, corresponding to the loss of Cd from the water column to the sediments in treatment 1 over the same time period (Fig. 4.4a). On day 0, particulate K_d^a in treatment 1 was considerably larger than to those in treatments 2-4, but by day 40 was similar to those in treatments 3 and 4 which had increased from day 0. At the same time, treatment 2 particulate K_d^a was somewhat elevated above those in all other treatments, although its associated SE was quite high. By day 80, treatments 2-4 particulate K_d^as were larger than that in treatment 1. The apparent partitioning coefficient for the sediment in treatment 1 was consistently larger than those in treatments 2-4 and increased over time. This is consistent with the greater

Table 4.3. Metal concentrations (μg g^{-1} dry wt.) in limnocorral sediments on day 0 and day 80 compared to background sediment metal concentrations in Roddy Lake (background) and target metal concentrations. Values are means ± SE.

			Metal concentration μg·g^{-1} dry wt.			
Metal	Background	Treatment	day 0	day 40	day 80	Target
Cd	0.2 ± 0.03	1	1.4 ± 0.1	1.7 ± 0.1	1.8 ± 0.03	1.5
		2	1.0 ± 0.1	2.1 ± 0.2	1.4 ± 0.2	1.5
		3	1.1 ± 0.1	1.4 ± 0.1	1.4 ± 0.02	1.5
		4	1.0 ± 0.1	1.3 ± 0.1	1.7 ± 0.2	1.5
Cu	0.6 ± 0.07	1	0.7 ± 0.2	0.7 ± 0.05	0.9 ± 0.1	Background
		2	1.0 ± 0.2	1.7 ± 0.3	1.4 ± 0.4	1.6
		3	1.1 ± 0.1	1.8 ± 0.1	2.1 ± 0.2	2.2
		4	1.9 ± 0.2	3.2 ± 0.3	3.6 ± 0.6	3.8
Zn	5.3 ± 0.8	1	7.5 ± 0.2	6.6 ± 0.2	6.3 ± 0.9	Background
		2	11.1 ± 1.2	18.0 ± 2.1	12.3 ± 2.9	16
		3	13.7 ± 1.4	18.5 ± 0.8	20.3 ± 1.1	21
		4	17.3 ± 1.0	27.2 ± 2.0	34.3 ± 4.5	37
Pb	1.8 ± 0.04	1	1.5 ± 0.1	2.0 ± 0.2	1.9 ± 0.1	Background
		2	3.6 ± 0.3	9.5 ± 1.4	5.3 ± 1.6	5.4
		3	5.3 ± 0.8	9.0 ± 0.6	10.2 ± 1.8	7.2
		4	9.3 ± 1.6	17.3 ± 1.1	23.1 ± 5.6	13
Ni	1.0 ± 0.4	1	0.9 ± 0.04	1.0 ± 0.1	1.3 ± 0.2	Background
		2	1.9 ± 0.3	2.9 ± 0.7	2.5 ± 0.7	3.1
		3	1.8 ± 0.3	3.3 ± 0.2	3.8 ± 0.2	4.1
		4	2.5 ± 0.3	4.6 ± 0.3	6.5 ± 0.9	7.2

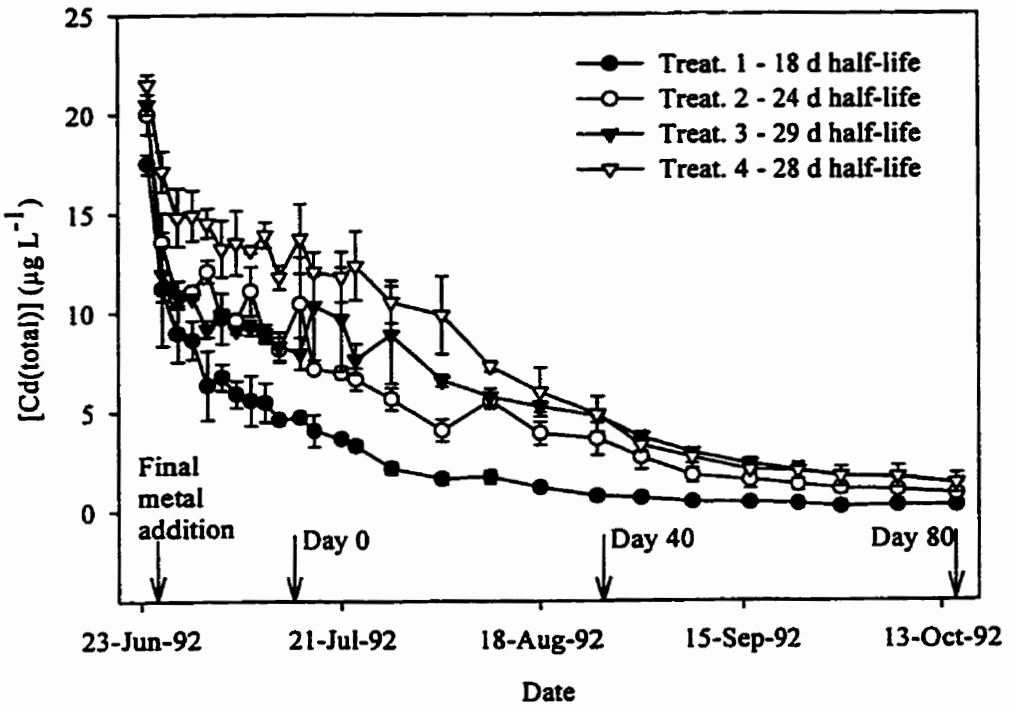


Figure 4.2. Loss of Cd from the water column after the final metal addition. Values are mean concentrations for each treatment (±SE, n=2).

Table 4.4. Metal concentration ($\mu g\ L^{-1}$) in unfiltered water on day 0, 40 and 80. Values are means ± SE (n=2)., except pH (n=1).

Treatment	Day	pH	Cd	Cu	Zn	Pb	Ni
Roddy Lake	0	7.13	<0.02	<0.5	0.63	<0.3	<0.5
1	0	6.89	4.61 ± 0.02	<0.5	3.82 ± 2.06	<0.3	<0.5
2	0	7.04	9.30 ± 0.87	11.9 ± 1.6	128 ± 8	29.9 ± 5.4	18.5 ± 0.7
3	0	6.82	7.95 ± 0.81	17.4 ± 1.0	209 ± 14	46.3 ± 1.8	24.6 ± 0.7
4	0	6.78	13.7 ± 1.7	30.8 ± 0.6	414 ± 4	87.0 ± 2.0	56.7 ± 5.4
Roddy Lake	40	7.27	<0.02	<0.5	1.55	0.52	<0.5
1	40	7.33	0.66 ± 0.14	<0.5	1.19 ± 0.08	<0.3	<0.5
2	40	7.02	3.20 ± 0.63	4.55 ± 0.47	38.0 ± 5.4	4.38 ± 0.62	6.15 ± 1.06
3	40	7.49	4.34 ± 0.17	6.7 ± 0.34	78.9 ± 2.7	7.41 ± 0.57	12.5 ± 0.8
4	40	7.56	4.00 ± 0.17	10.7 ± 0.4	155 ± 21	12.1 ± 1.5	21.9 ± 0.7
Roddy Lake	80	6.74	<0.02	0.72	1.40	0.93	<0.5
1	80	7.49	0.71± 0.02	<0.5	2.50 ± 1.69	<0.3	<0.5
2	80	6.77	1.02 ± 0.09	2.97 ± 0.06	23.4 ± 1.1	1.30 ± 0.003	4.03 ± 0.31
3	80	7.59	1.66 ± 0.03	4.12 ± 0.17	47.6 ± 1.0	1.68 ± 0.26	7.59 ± 0.23
4	80	7.43	1.60 ± 0.50	5.19 ± 0.74	82.6 ± 19.3	2.49 ± 0.95	11.2 ± 1.6

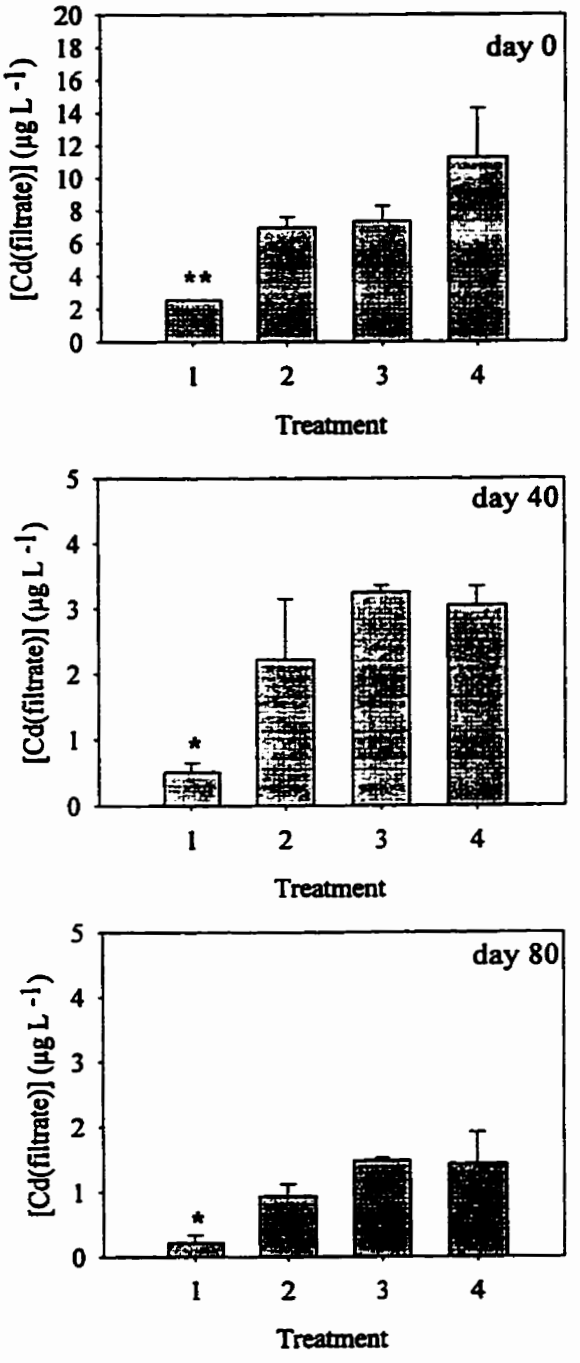


Figure 4.3. Cd concentration (µg L^{-1}) in <1 µm filtrate on day 0, 40 and 80. Values are means (±SE, n=2). Treatment 1 is significantly different from the other treatments at * - P<0.05, ** - P<0.01.

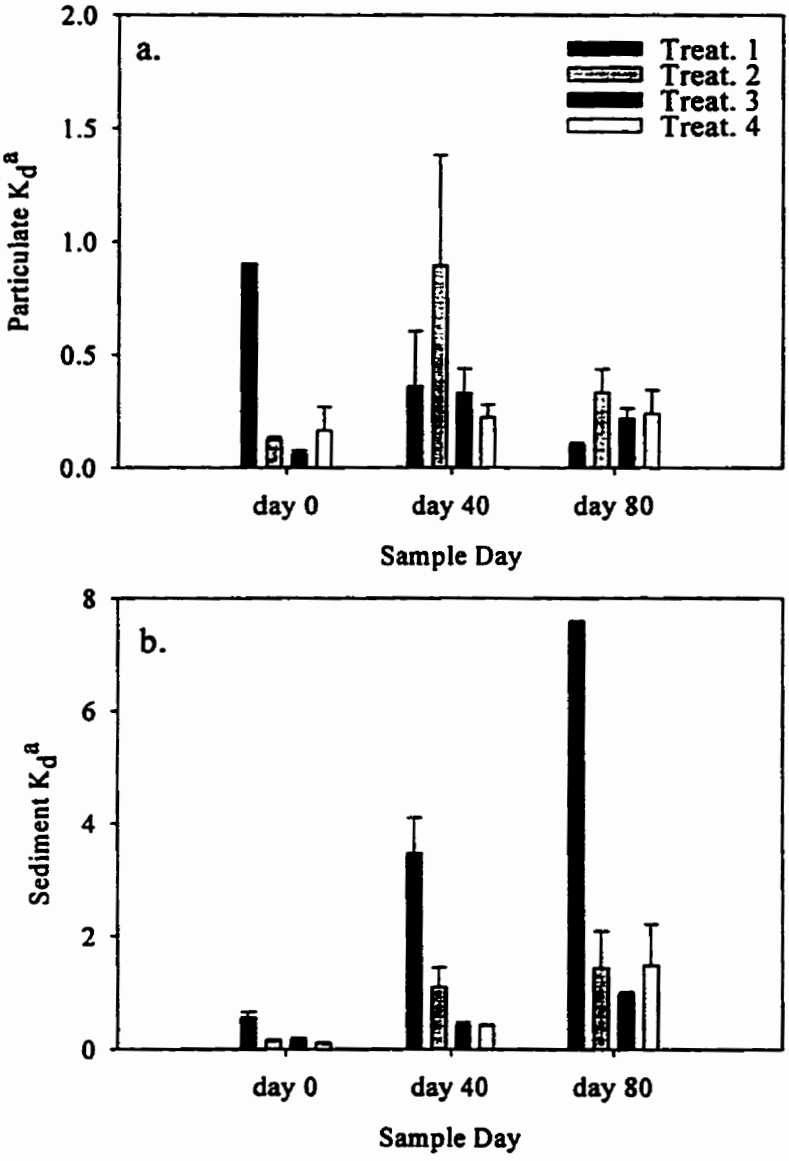


Figure 4.4 Apparent partitioning coefficients for Cd in each treatment on each sample day. a. particulate K_d^a. b. sediment K_d^a. The particulate K_d^a for treatment 1 on day 0 has an n=1.

loss of Cd from the water column and its sorption onto the surface sediment in treatment 1 relative to treatments 2-4 (Fig. 4.4b).

Condition of the mussels

Transplanting mussels from Lake 104 to the cage in Roddy Lake for 80 days did not significantly affect condition (0.183 in Lake 104 in October vs. 0.168 in Roddy Lake in October) (Table 4.5). Enclosure in the limnocorrals for 80 d, nevertheless, was associated in some cases with a decline in condition (Table 4.5). For example, 0-80 d mussels in treatment 1 and 4, were in significantly poorer condition than caged mussels ($F_{5,1}$=11, P=0.04). This difference between mussels in the limnocorrals and the cage was not seen in the 40-80 d mussels. The longer the mussels were held in the limnocorrals, the lower was the condition. For example, treatment 4, 0-80 d mussels were lower in condition than treatment 4 40-80 d mussels (F_5=9, P=0.03). Nevertheless, condition did not vary among the 4 treatments for either 0-80 d or 40-80 d mussels.

Accumulation of Cd by mussels

Caging mussels in Roddy for 80 d did not result in a change in whole body cadmium concentrations ([Cd(body)]) compared with Lake 104 mussels on the same day (1.62 μg g dry wt^{-1} vs. 1.29 μg g dry wt^{-1}) (P>0.05).

Treatment had a significant effect on Cd concentrations in 0-80 d mussels ($F_{3,3}$=65, P<0.005) (Fig. 4.5). The highest [Cd(body)] were in mussels from treatment 2 (lowest concentration of metal mixture) and 1 (Cd alone) followed by treatments 3 and 4. Among treatments with the metal mixture, [Cd(body)] declined with increasing addition of the metal mixture ($F_{3,3}$=57, P=0.005). As expected, the 40-80 d mussels, introduced into the limnocorrals on day 40, as expected accumulated significantly less Cd than the 0-80 d mussels (P<0.0001). Not surprisingly, treatment 1 mussels had the lowest [Cd(body)] of the treatments, since water column [Cd] in treatment 1 was lower than in other treatments on day 0 and day 40 when the mussels were introduced into the limnocorrals (P<0.005).

Table 4.5. Condition indices of mussels collected in October 1992 from treatments and cage in Roddy Lake and source Lake 104. Values are means ± SE. Values for Lake 104 and caged are a means of 5-6 animals each. Values for treatments are means of replicate treatments consisting of means of 5 animals in each.

	Condition index		
	Fresh day 80	0-80 d	40-80 d
Lake 104	0.183 ± 0.03	-	-
Cage	-	0.168 ± 0.01	0.182 ± 0.03
Treatment 1	-	0.129 ± 0.01	0.179 ± 0.01
Treatment 2	-	0.146 ± 0.01	0.155 ± 0.01
Treatment 3	-	0.146 ± 0.001	0.189 ± 0.02
Treatment 4	-	0.127 ± 0.01	0.193 ± 0.01

Expressing metal levels as body burdens, which adjusts for variable soft-tissue weights, resulted in fewer statistical differences among treatments. Nevertheless, whole-body Cd burdens among treatments showed the same trends as [Cd(body)] (Fig. 4.4).

Like whole body concentrations and body burdens, tissue [Cd] were dependent on treatment (Fig. 4.6). The highest [Cd] were generally found in the gill followed by kidney and mantle. In the gill, [Cd] were highest in mussels from treatment 1 and decreased with increasing addition of the metal mixture. This trend was also observed for [Cd] in the foot. In the kidney, Cd levels were similar in treatments 1 and 2 and lower in treatments 3 and 4. In the mantle, [Cd] decreased from treatment 2 to 4 with the increasing concentration of the metal mixture. There were no obvious differences among treatments in viscera [Cd].

Accumulation of Cu, Zn, Pb and Ni

Mussels accumulated substantial amounts of Cu, Zn, Pb and Ni in treatments with the metal mixture (Fig. 4.7). Two different accumulation patterns among treatments

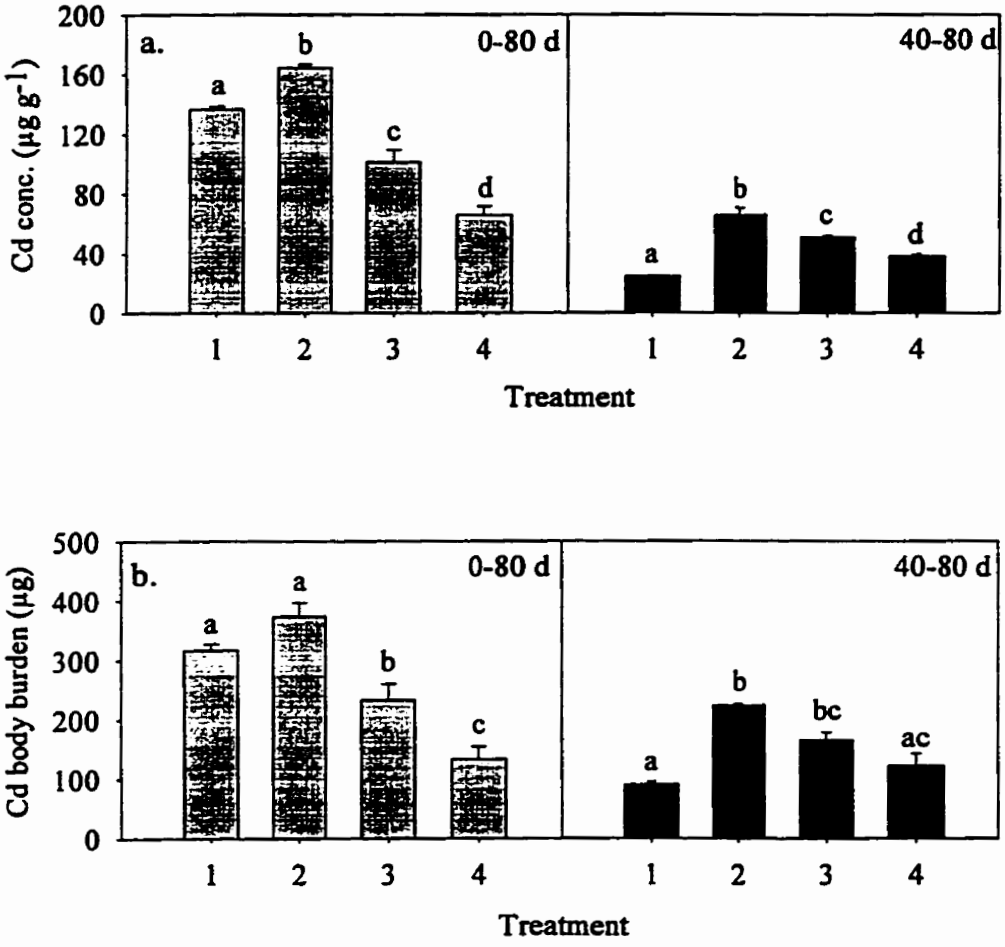


Figure 4.5. Cd in whole bodies of 0-80 d and 40-80 d mussels. Bars are means (±SE, n=2) for each treatment. a. Concentration (µg g^{-1}). b. Body burden (µg). Bars with the same letter are not different at the P=0.05 level (0-80 d and 40-80 d mussels are statistically separate).

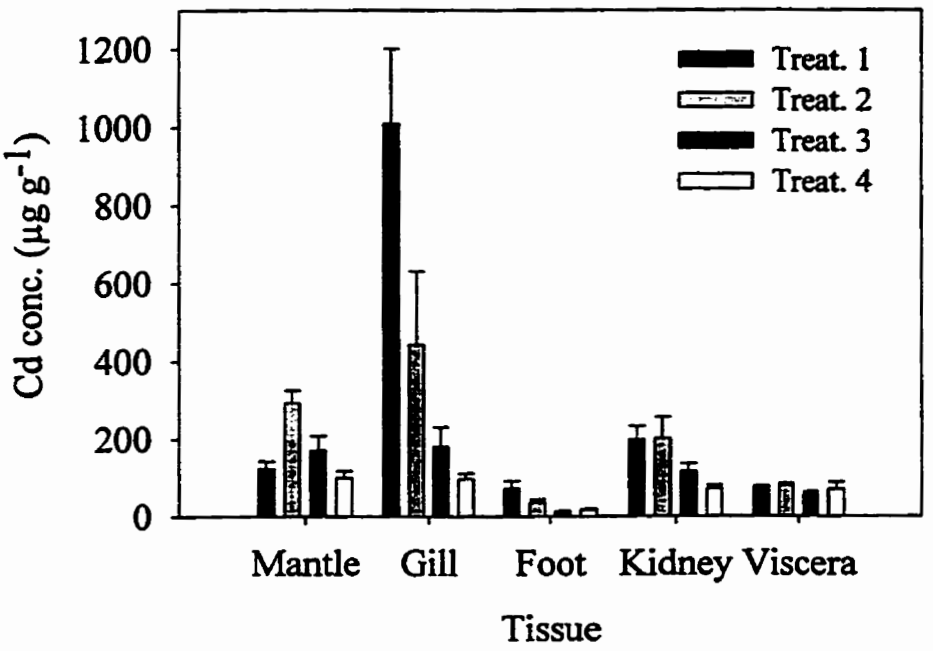


Figure 4.6. Cd concentrations (µg g^{-1}) in five tissues of 0-80 d mussels. Bars are means (±SE) of 5 animals in each treatment.

were found for Cu and Zn, and Pb and Ni. Whole-body [Cu] and [Zn] in 0-80 d mussels did not reflect the proportional increase in metals added to the limnocorrals. Similar accumulation patterns were observed for Cu and Zn where metal concentrations in treatment 2 in 0-80 d mussels were marginally higher than in treatments 3 and 4 (Fig. 4.7a and b). However, whole-body [Cu] and [Zn] in 40-80 d mussels were lower in treatment 2 than in treatments 3 and 4, reflecting the increasing metal additions (Cu, $F_{3,3}$=158, P<0.001; Zn, $F_{3,3}$=197, P<0.001). Lead accumulation in 0-80 d and 40-80 d mussels and Ni accumulation in 0-80 d mussels reflected the increasing metal additions among treatments. Whole-body [Pb] in treatment 4 mussels were significantly higher than treatment 2 mussels (0-80 d - $F_{3,3}$=348, P<0.0005; 40-80 d - $F_{3,3}$=69, P<0.005) (Fig. 4.7c). Nickel concentrations in 0-80 d mussels were significantly higher in treatment 4 mussels compared to treatment 2 ($F_{3,3}$=121, P<0.005), but no differences were found among treatments in 40-80 d mussels (Fig. 4.7d).

Metallothionein concentrations in mussels

Exposure of mussels to all four treatments generally resulted in elevation of [MT] above levels found in caged mussels (Fig. 4.8). Metallothionein concentrations were highest in the kidney, followed by the mantle, gill and viscera. In the kidney, foot, and viscera, [MT] tended to be highest in treatments 1 and lowest in treatment 4, following the pattern of [Cd] in the tissues. Gill [MT] was elevated in treatment 3 relative to the other treatments and did not reflect [Cd] in the gill. Metallothionein concentrations in the calculated whole body were lowest in treatment 4 and increased from treatment 1 to 3. Metallothionein and metal concentrations (Cd, Cu, Zn, or Cd+Cu+Zn; nmol g dry wt^{-1}) for individual tissues or the whole body were not correlated (P>0.05).

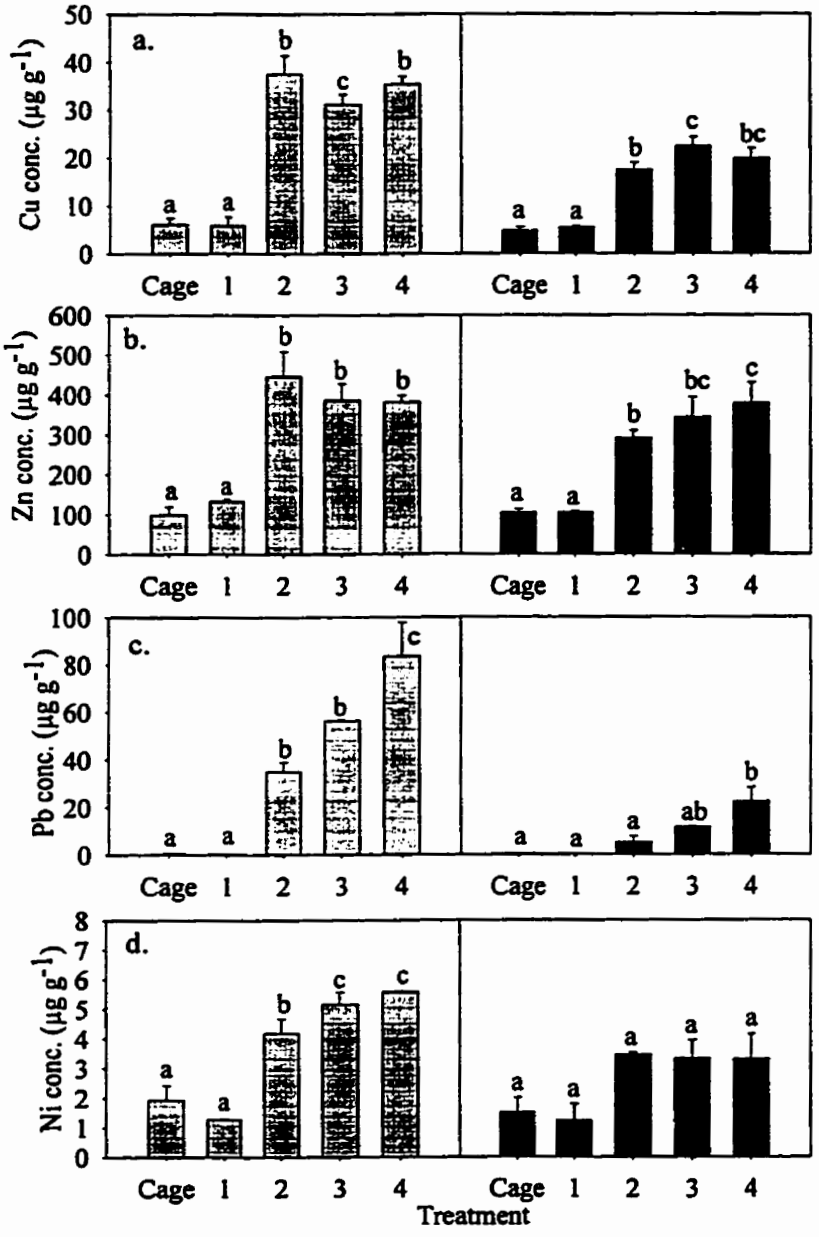


Figure 4.7. Concentrations (µg g^{-1}) of other metals in whole bodies of 0-80 d (grey bars) and 40-80 d (black bars) mussels. Bars are means (±SE, n=2) for each treatment. Bars with the same letter are not different at the P=0.05 level (0-80 d and 40-80 mussels are statistically separate). a. Cu. b. Zn. c. Pb. d. Ni.

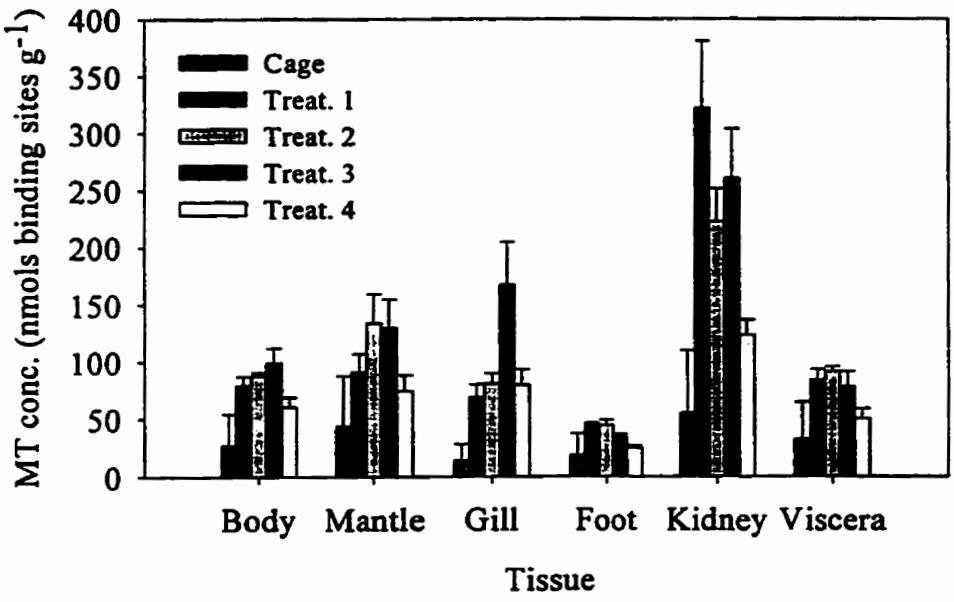


Figure 4.8. Metallothionein concentrations in five tissues of 0-80 d mussels and calculated for whole body. Bars are means (±SE) for 5 animals in each treatment.

4.5 Discussion

Partitioning of Cd in water column

The presence of the metal mixture had a significant effect on the partitioning of Cd in the water, resulting in an increased residence time for Cd in the water column and higher concentrations in the water at each sampling. Radioisotope studies performed at the ELA using shallow limnocorrals showed that metals are removed from the water column by two primary mechanisms: 1) adsorption to particulate material and subsequently settling to the bottom of the limnocorrals, and 2) adsorption directly to sediments after diffusion through a stagnant film (diffusive sublayer) overlaying the sediments (Santschi et al. 1986). Both removal processes may have been influenced by some form of competition among metals for binding sites resulting in longer residence time for Cd in the water column. For example, the lower particulate K_d^s in treatments 2 to 4 on day 0 suggests that there was less Cd adsorbed onto particles in the presence of other metals, thus limiting the removal of Cd from the water column by settling particles. Furthermore, the distinctly lower sediment K_d^s in treatments with the metal mixture strongly suggests that binding sites on the surface sediment (~top 1 mm) may have initially been saturated by the metals leading to increased competition and decreased adsorption. Santschi et al. (1986) found that the apparent Kd for ^{59}Fe adsorption on suspended particles was smaller in the limnocorral which received an addition of stable Fe, resulting in a slower removal of Fe from the water column. The authors suggested that the increased Fe loading saturated the adsorption capacity of the particles. Despite the possibility of initial saturation of surface sediments in this experiment, it is unlikely that the sediments were completely saturated and unable to absorb more metal because by day 80 [Cd] in sediment were similar among treatments. Over time, metal-laden particles move deeper into sediments by physical processes and molecular diffusion opening up new binding sites at the sediment-water interface (Santschi et al. 1986).

Various metals are thought to sorb predominantly to different molecular binding sites, but some sites may be mutually shared and competition for these sites results (Sigg et al. 1987). For example, Benjamin and Leckie (1980) found that Zn had a greater effect than Cu or Pb on Cd adsorption to oxides suggesting that Cd and Zn may have some

shared binding sites. On a molar basis, there was five to seven times more Cu, Pb and Ni and 43 times more Zn than Cd added to the limnocorrals in the present study. The high ratio of Zn to Cd makes it likely that Zn had the greatest impact on the sorption of Cd to particles, although the present experiment was not designed to look at interactions among individual metals.

Competition from Ca /Mg has also been shown to decrease Cd adsorption onto particulate matter (Cowan et al. 1991; Laxen 1985). Cowan et al. (1991) showed that Ca (2.5 mM) reduced the sorption of Cd (1 μM) onto amorphous iron oxyhydroxide, due to competition for mutually accessible surface sites. Further, Ca concentrations as low as 0.25 mM were also found to cause the desorption of Cd from amorphous iron oxyhydroxide. Chemical analysis of the water showed that Ca increased with metal addition on day 0, 40 and 80 (0.06 mM in treatment 1 vs. 0.1 mM in treatment 4 on day 80).

Differences in pH among the treatments (6.78-7.04) on day 0 were insignificant compared to the large differences in water column [Cd] among treatments and probably did not influence the partitioning of Cd in the water column. Furthermore, higher pH in treatments 3 and 4 relative to treatments 1 and 2 on day 40 should have resulted in a higher particulate K_d^s, which did not happen (see Fig. 3). Wagemann et al. (1994) report a significant shift of Cd from Cd^{2+} to particulate-bound Cd at pH > 6.5, where the percentage of particulate-Cd exceeds Cd^{2+} at around pH 7.4 (for 1.0 mg suspended sediment L^{-1}).

The addition of the metal mixture along with Cd, resulted in a decrease in the loss rate of Cd from the water column and a greater opportunity for uptake by the mussels. It is important to note that the sediments used in this study had very low background metal concentrations and that more contaminated sediments nearing saturation may have resulted in an even greater effect of the metal mixture on Cd partitioning in the limnocorrals.

Effect of metals and enclosure on mussel condition

When using transplanted and caged mussels in metal uptake studies, it is important to consider how these factors might have affected the overall health of the

mussels and consequently their tissue metal concentrations. It was shown that the condition of mussels transplanted and caged outside the limnocorrals for 80 days did not significantly differ from the condition of mussels from the source lake. However, mussels held in the limnocorrals showed a time dependent decrease in condition, possibly due to exposure to toxic metal concentrations. The water column concentrations for Cd and Zn were at or above 96-h LC50 values determined for a related species, juvenile *Anodonta imbecilis*, exposed separately to Cd (9 $\mu g\ L^{-1}$), and Zn (268 $\mu g\ L^{-1}$) (Keller and Zam, 1991). Keller and Zam (1991) also list the 96-h LC50s for several bimetallic combinations which generally increased Cd's 96-h LC50 and decreased the 96-h LC50 for Zn, Cu and Ni. As a mixture in treatment 4, Cu and Zn were at lethal levels and Cd was near lethal levels and may have caused the observed loss in mussel condition and mortalities. However, the loss of condition in treatment 4 mussels was only slightly greater than in mussels in treatment 1, not exposed to toxic metal concentrations. The lack of a significant difference in condition among treatments in either exposure group (0-80 d or 40-80 d) suggests that the length of enclosure played the more important role in the loss of condition in the mussels. Loss of condition may be attributed to a variety of factors including metal toxicity and the abundance and nutritional quality of suspended particles filtered by the mussels. Given that the control cage (exchanges with lake water) was not a true control for the enclosure effect of the limnocorral (no exchange with lake water) it is not possible to distinguish between the effects of the enclosure and metals on mussel condition.

One of the consequences of lower condition in mussels is artificially inflated tissue metal concentrations. By expressing metal accumulation on a content basis which controls for differences in soft tissue weights, treatment groups may be more accurately compared. In the present study, similar trends among treatments were found for whole-body and tissue metal expressed as concentrations (Cd (Fig. 4.5), Cu, Zn, Pb and Ni) and contents. In contrast, a strong decreasing trend in tissue mercury concentrations observed in *Elliptio complanata* caged along a mercury contamination gradient along the Sudbury River, Massachusetts was less evident when the mercury levels were expressed on a content basis (Salazar et al. 1996).

Effect of the metal mixture on Cd accumulation

Despite the high water column Cd concentrations in the presence of the mixture, Cd accumulation in the mussels was reduced with increasing concentrations of the metal mixture. This same trend was observed in 40-80 d mussels exposed to lower overall metal concentrations (70-90% lower, see Table 4.4)). This experiment was not designed to distinguish the effects of individual divalent metals in the mixture on Cd accumulation. Nevertheless, the results of this experiment show similarities with other studies that examine the interactions between Cd and one or two of the metals in the mixture. Hemelraad et al. (1987) exposed the freshwater mussel, *Anodonta cygnea*, to Cd (25 μg L^{-1}) and to a mixture of Cd (25 μg L^{-1}) and Zn (2.5 mg L^{-1}), in the laboratory for 16 weeks, without food. In the presence of Zn, Cd accumulation of was reduced by 50% in the whole animal and by over 50% in the gills and mantle and was not affected in the kidney. A similar reduction in Cd accumulation was observed in *Mytilus edulis* (whole body) exposed in a laboratory factorial experiment to Cu (20 μg L^{-1}) and Zn (200 μg L^{-1}) (Elliott et al. 1986). At lower Zn (100 μg L^{-1}) and Cd (10 μg L^{-1}) exposure concentrations, Cd accumulation in the mussels was slightly enhanced. In the present limnocorral experiment, Cd accumulation at the lowest concentration of the metal mixture was not significantly different from that in the absence of the mixture, but at higher mixture concentrations in treatments 3 and 4, Cd accumulation was reduced by 26 and 50% in the whole body and by 42 and 64 % in the kidney, respectively. Reduced uptake in the gill was observed at all concentrations of the metal mixture and ranged from 56 to 91%.

An alternative explanation for the observed reduction in Cd uptake at higher concentrations of the metal mixture is that the mussels spent more time with the valves closed. Reducing the periods of valve openness and time actively filtering might be expected to reduce metal uptake. Salánki and V.-Balogh (1989) found a decrease in the duration of active filtration periods in the unionid mussel *Anodonta cygnea* from 20 h to 8 h and 30-60 h to 7 h during a 240 h exposure to 10 μg L^{-1} Cu and 50 μg L^{-1} Pb, respectively. The effect of reduced filtration activity on metal uptake in mussels is not well understood at this time. Pynnönen (1995) found that [Cd] in the gill of *Anodonta cygnea* exposed in a laboratory for 2 wk were less after exposure to 200 μg L^{-1} Cd than

exposure to 50 μg L^{-1} Cd. Further studies identified a positive correlation between filtration activity and Cd accumulation in *Anodonta cygnea* exposed to 50 μg L^{-1} (K. Pynnönen, Department of Zoology, University of Helsinki, Helsinki, Finland, personal communication). In contrast, an experiment that studied the effects of Cd, Cu and Zn interactions on the filtration rate of the zebra mussel *Dreissena polymorpha*, found that Cd was accumulated at all concentrations (10 - 1000 μg L^{-1}), despite up to an 80% decrease in filtration rate (Kraak et al. 1994).

Based on the concentration ranges of these studies, a decrease in active filtration periods of *P. grandis* might have occurred, resulting in the observed decrease in metal uptake at the highest concentrations of the metal mixture. If so, then a corresponding decrease in condition relative to treatment level would also be expected due to a decrease in the filtration of food particles, but this was not observed. Furthermore, reduced uptake of all the metals at higher concentrations of the metal mixture would have been expected and this was not observed (Fig. 4.6). For example, a treatment-dependent increases in Pb and Ni were observed in 0-80 d mussels. Similar concentrations of Cu and Zn were observed among the treatments, but their essential role in basic mollusc physiology dictates that the uptake of these metals could have been regulated. The effect of reduced filtration activity on metal uptake has great consequences for the use of mussels in bioaccumulative monitoring programs, thus a better understanding of this relationship is needed. Specifically, studies are needed that use environmentally-relevant metal concentrations and chronic exposure periods (weeks) that link thresholds for changes in filtration activity to impacts on metal uptake.

The soft-tissue metal concentrations observed in this study were similar to values obtained for *P. grandis* in mining areas. Cadmium concentrations in the treated 0-80 d and 40-80 d mussels ([Cd(body)] 24 - 153 μg g^{-1}) were in the range of resident *P. grandis* ([Cd(body)] 19 - 129 μg g^{-1}) collected along a metal contamination gradient in the Rouyn-Noranda mining region, northwestern Quebec (Couillard et al. 1993). However, Cd levels in the gill of 0-80 d mussels from treatments 1 and 2 (400 - 1000 μg g^{-1}) were nearly 4 times higher than those measured in *P. grandis* collected from Rouyn-Noranda (38 - 270 μg g^{-1}).

Effect of the metal mixture on MT development

Exposure to Cd along with the metal mixture resulted in the induction of MT proteins, with the highest levels found in the kidney. Malley et al. (1993) found a similar distribution of MT among body parts in *P. grandis* from pristine Lake 377 and from Lake 382 receiving experimental Cd additions in the ELA where [MT] were in the following decreasing order: kidney > viscera > gill > foot > mantle. Couillard et al. (1993) also report lower MT levels in the gills relative to the hepatopancreas of *P. grandis* sampled along a contamination gradient in the mining area of Rouyn-Noranda, Quebec.
It is difficult to conclude from these results the effect of the metal mixture on Cd at the subcellular level, due to variable [MT] among individuals, small sample size and absence of HPLC data on Cd partitioning. The similar trend in [MT] and [Cd] in the kidney, foot and mantle among treatments suggests that the metal mixture did not have any further effect on Cd at the subcellular level. Metallothionein induction in freshwater mussels has been shown to be highly specific to Cd even in the presence of high levels of Cu and Zn (Couillard et al. 1993).

Competition in the environment and on biological surfaces

This experiment was designed to examine the effects of a metal mixture on Cd partitioning in the aquatic environment and on Cd uptake in freshwater mussels. The partitioning of Cd in the water, on particles and sediment over the course of the experiment suggested that the metal mixture slowed the removal of Cd from the water column possibly through competition for sites on settling particles and on the surface sediment. This resulted in more Cd in the water column available for uptake by the mussels in the presence of the mixture. Nevertheless, the higher water column [Cd] did not result in increased accumulation of Cd by the mussels. It appeared that some factor related to the mussel and not to the distribution of Cd in the environment was determining uptake.

If valve closure and reduced filtration activity were not determining factors, then there are two possible explanations for these results, depending on the primary routes of uptake for Cd in freshwater mussels. If mussels accumulate a large portion of metal from particles or "food", then the reduction in particulate bound Cd at the highest

concentration of the metal mixture, indicated by the lower particulate K_d^a, may have limited Cd uptake. Alternatively, if the mussels accumulate the majority of their metal burden from the water, then direct competition among metals at sites of uptake on the mussels might have reduced Cd uptake. Recent studies by Wang et al. (1996) using the marine mussel *Mytilus edulis* suggest that Cd is accumulated from both dissolved and particulate pathways, but that the largest portion of Cd is obtained from dissolved sources (>50 - 80%). Uptake of Cd by aquatic organisms in freshwater environments occurs either by binding to transport ligands that traverse the membrane and deposit the metal in the interior of the cell or by incorporation of the metal into an active pump for a major ion (Rainbow and Dallinger 1993). Competition for membrane transport ligands from the metal mixture or for active pumps by elevated Ca concentrations may have limited Cd entry into the mussels.

Environmental Realism

Given that one of the objectives of this experiment was to add an element of ecological realism to metal mixture studies, it seems appropriate to evaluate the results based on those merits. In the present experiment, concentration ratios between Cu, Zn, Pb and Ni held constant between treatments, were based on naturally occurring ratios of these elements at the experimental site. In comparison to metal levels near base-metal smelters, the target sediment concentrations were relatively low (Harrison and Klaverkamp, 1990), and initial water column metal concentrations in the limnocorrals during the experiment were relatively high (Nriagu et al. 1982; Couillard et al. 1993). For example, the highest [Cd], [Cu] and [Zn] in surface water in mining region of Rouyn-Noranda, Quebec were approximately 35, 3 and 26 times less than the highest levels used in the present experiment. However, the water column metal concentrations at their highest levels were considerably lower than the limits listed under the Metal Mining Liquid Effluent Regulations for Cu (300µg L^{-1}, monthly arithmetic mean), Zn (500 µg L^{-1}), Pb (200µg L^{-1}) and Ni (500 µg L^{-1}) (AQUAMIN, 1996).

4.6 Conclusions

The effect of a metal mixture on the partitioning of Cd in water and sediment and its uptake by freshwater mussels was examined in a limnocorral experiment. The metal mixture resulted in longer residence times for Cd in the water, but reduced Cd accumulation in the mussels. It is hypothesized that competition for binding sites on suspended particles and surface sediment and at sites of uptake on the mussels was responsible for the effects of the metal mixture. The effect of the metal mixture on the partitioning of Cd in the water column may have been less important than the effect of the metal mixture at uptake sites on the mussel in determining Cd accumulation in the mussels. Mussel behavior (valve closure and reduced filtration activity) may also have had a role in limiting Cd uptake in the presence of the metal mixture.

CHAPTER 5. THE EFFECT OF A METAL MIXTURE (Cu, Zn, Pb AND Ni) ON CADMIUM PARTITIONING IN LITTORAL SEDIMENTS AND ITS ACCUMULATION BY THE FRESHWATER MACROPHYTE *ERIOCAULON SEPTANGULARE*

5.1 Abstract

The effect of a metal mixture (Cu, Zn, Pb and Ni) on Cd fractionation in sediment and its accumulation by the freshwater macrophyte *Eriocaulon septangulare*, was examined in an *in situ* littoral experiment at the Experimental Lakes Area, Northwestern Ontario. Fresh sediment was spiked with Cd alone and together with the metal mixture at 3 increasing concentration levels. Macrophytes were planted in the spiked sediment and placed at 0.5 m in the littoral zone. The distribution of Cd among sediment fractions (easily-reducible (ER), reducible (R-ER) and organic (ORG)), porewater and macrophytes was determined biweekly for 10 weeks. The metal mixture had a significant effect on the distribution of Cd among geochemical fractions after 2 and 8 weeks, but not after 10 weeks. At the highest concentration of the metal mixture, Cd shifted from the ER (Mn-oxides) fraction onto the R-ER (Fe-oxide) fraction. Cd was accumulated by the shoots (<2.6 $\mu g\ g^{-1}$ dry wt.) and roots (<45 $\mu g\ g^{-1}$ dry wt.) of *E. septangulare*, and was still increasing after 10 weeks. Significantly higher Cd concentrations were found in the shoots of plants in the treatment with Cd alone and the treatment with the highest concentration of the metal mixture. Approximately 57% and 35% of total Cd extracted from the roots and shoots, respectively, was adsorbed to the outside of the plants (removed by a Ti (III) reagent) and did not vary significantly among treatments.

5.2 Introduction

The distribution and toxicity of Cd in the aquatic environment are affected by pH, hardness, alkalinity, sulfate, and concentration of organic matter (Campbell and Stokes 1985; Stephenson and Mackie 1989; Wagemann et al 1994; Tessier et al. 1993). Attempts have been made to take these factors into account when water and sediment quality guidelines for Cd have been developed (Jaagumagi 1992; CCREM 1987). Nevertheless, trace metals may also affect the distribution and toxicity of Cd (Rule and Alden 1996a; Huebert and Shay 1992; Santschi et al. 1986) and their influence is not generally considered in guidelines. This influence may be of critical importance since Cd is primarily released into the environment in mixtures along with other trace metals through non-ferrous metal mining, smelting and refining processes, industrial effluents, and domestic sewage (Nriagu 1990; AQUAMIN 1996; Kelly 1988).

The study of the toxicity of metal mixtures has focused on aqueous exposures under controlled laboratory conditions (Naddy et al. 1995; Kraak et al. 1994; Huebert and Shay 1992; Enserink 1991; Keller and Zam 1991). Studies of this kind are helpful for understanding the effect of metal interactions on uptake and toxicity, but further information is needed on how metals interact to affect their distribution and bioavailability in the natural environment, and particularly in sediments.

Sediments can be both a sink and source of trace metals in aquatic ecosystems. Assessing the potential risks that sediment-based metals pose to aquatic organisms living both in, or on, sediment depends on their bioavailability (Luoma and Fisher 1997). Chemical extraction techniques that selectively partition metals into geochemical fractions have been useful in improving our understanding of metal bioavailability (Tessier et al. 1993; Jenne and Luoma 1977; Luoma and Bryan 1981). The distribution of metals among geochemical fractions in sediment depends on the relative affinity of the metals for each fraction, the solubility product (K_{sp}) of metals for each fraction, and the concentrations of all metals present (Rule and Alden 1996a). It is this latter effect which is being examined in this study.

Submerged aquatic macrophytes have recently been examined as potential bioindicators of trace metal contamination in the aquatic environment (St-Cyr and

Campbell 1994; St-Cyr et al. 1994). Isoetids are rooted macrophytes that possess an isoetoid growth form which is characterized by a short stem and a rosette of stiff leaves (Hutchinson 1975). Isoetids possess other characteristics that make them potential biological indicator species. They are widespread throughout eastern North America (Yan et al. 1985; France and Stokes 1988), abundant (Hitchin 1984), easy to collect and identify unequivocally, and they concentrate metals (Malley unpublished data; Yan et al. 1985). Their predominantly sediment-based nutrition makes them useful tools for studying the availability of contaminants from the sediment (Boston et al 1987), and recent studies have examined the relationship between metal concentrations in isoetids and total metal concentrations in sediment (Jackson et al. 1993; Jackson and Kalff 1993).

The present work examines the effect of a metal mixture on Cd partitioning in littoral sediment and on its accumulation by a macrophyte under field conditions. The objectives of this study were 1) to describe the effect of a metal mixture (Cu, Zn, Pb and Ni) on the partitioning of Cd into different geochemical sediment fractions; 2) describe Cd uptake in the roots and shoots of the isoetid *E. septangulàre*, and; 3) identify relationships between the bioavailability of Cd based on its partitioning into different geochemical sediment fractions and uptake by *E. septangulàre*.

5.3 Materials and Methods

Experimental site

The site of the *in situ* exposure experiment was a shallow, protected bay in Roddy Lake (93 43' W, 49 41' N) which is located at the Experimental Lakes Area (ELA) (Fig. 5.1 and described by Stewart (under review)). The ELA is a research preserve located at 52 km southeast of Kenora, Ontario on the southwestern part of the Precambrian Shield (Brunskill and Schindler 1971). Water chemistry from Roddy Lake is shown in Table 5.1.

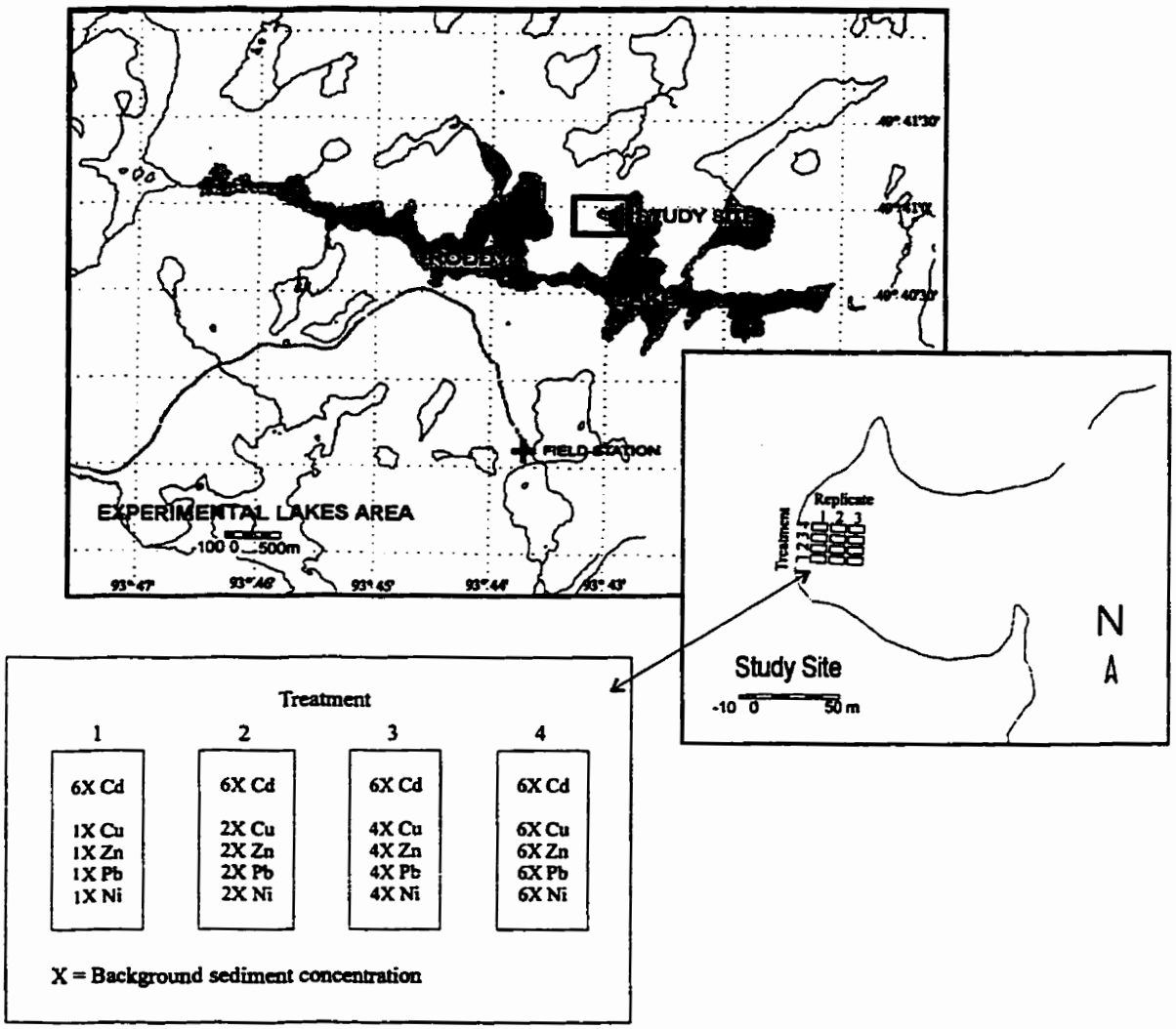


Figure 5.1. Location of study site in Roddy Lake at the Experimental Lakes Area. Location of plant trays and experimental design is also shown.

Table 5.1. Surface water chemistry of Roddy Lake and Lake 104. Values for Roddy Lake are means (±SD) of 6 samples from 8 June to 15 October 1992. Values for Lake 104 are based on a single sample taken on the 15 September 1992. Table is from Stewart (under review).

Parameter	Roddy Lake	Lake 104
NH_4-N, μg L^{-1}	9.7 ± 4.0	9
DIC, μmol L^{-1}	143 ± 8	120
DOC, μmol L^{-1}	413 ± 23	940
Na^+, mg L^{-1}	1.00 ± 0.05	0.98
K^+, mg L^{-1}	0.43 ± 0.07	0.44
Ca^{2+}, mg L^{-1}	2.41 ± 0.12	2.53
Mg^{2+}, mg L^{-1}	0.68 ± 0.02	0.68
Fe, mg L^{-1}	0.02 ± 0.01	0.14
Mn, mg L^{-1}	0.01 ± 0.0	0.01
Cl^-, mg L^{-1}	0.37 ± 0.02	0.24
SO_4, mg L^{-1}	3.23 ± 0.09	1.64
Alkalinity, μeq L^{-1}	134 ± 4.7	123
pH	6.74 - 7.27[a]	6.59
O_2, mg L^{-1}	9.15 ± 0.41	8.7

[a] Range

Experimental Design

The experiment consisted of four treatments with three replicates (n=3) per treatment, for a total of 12 experimental units (Fig 5.1). The experiment was designed to raise Cd sediment concentrations by 6 times above background in Treatment 1. Treatments 2, 3 and 4 were to receive a mixture of Cu, Zn, Pb and Ni to raise sediment metal concentrations by 2, 4 and 6 times above background, respectively (Table 2). The target metal concentrations in sediments are at the lower range of those observed in areas receiving atmospheric deposition from mining and smelting processes (Campbell et al. 1985; Couillard et al. 1993).

Sandy, littoral sediment (top 4 cm, <2 m water depth) collected from the site in Roddy Lake on 8 July 1995 was mixed in a 40-gallon cement mixer and separated into 4 homogeneous batches each ~16 kg. Each batch was then returned to the mixer and spiked according to the 4 treatment levels on 10 July (Table 2). Metals were added to the sediments as salts ($CdCl_2 \cdot 2½\ H_2O$, $CuCl_2 \cdot 2\ H_2O$, $ZnCl_2$, $Pb(NO_3)_2$ and $NiCl_2 \cdot 6\ H_2O$; Fisher Scientific, ACS grade) dissolved in 250 ml distilled deionized water and mixed for 5 minutes. Spiked sediment from each of the 4 treatment batches was divided into three replicate batches and placed in acid-washed polyethylene pails which were stored on the shore and allowed to equilibrate for 3 days. Post-spike samples were taken from each replicate batch one day after the metal spike on 11 July for analysis. Individual batches of sediment were then placed in 1 ft (0.3 m) wide x 2 ft (0.6 m) long x 2 in (5 cm) deep plastic potting trays prior to the introduction of the plants.

Nearby ELA Lake 104 (93 50' W, 49 41' N) was the source of the macrophyte *E. septangulàre* With. for planting in the spiked sediment. *Eriocaùlon septangulàre* has been successfully transplanted as part of other experiments at the ELA (Howell 1990). Water chemistry for Lake 104 is given in Table 5.1. Lake 104 is a brown water lake with higher DOC and slightly lower pH than Roddy, but does not constitute a major change of water chemistry for the plants. On 11 July plants were collected from Lake 104 from water depths <2 m, placed in coolers and transported to Roddy Lake. The plants were agitated in lake water to remove loosely attached sediment and debris and then planted in the trays of spiked sediment. On 13 July (day 0) after the introduction of the plants the trays were placed on the bottom in Roddy Lake at 0.5 m water depth and sampled after 2

Table 5.2. Target, post-spike and 10 week sediment metal concentrations (μg g^{-1} dry wt.) in 4 treatments. Values are mean (n=3) acid-extracted metal concentrations. Values in parentheses are factors by which the sediments were to be increased over background levels.

	Metal				
Treatment	Cd	Cu	Zn	Pb	Ni
Background	0.15 ± 0.008	0.54 ± 0.02	5.33 ± 0.67	1.57 ± 0.09	1.86 ± 0.10
Target					
1	0.90 (6X)	(1X)	(1X)	(1X)	(1X)
2	0.90 (6X)	1.08 (2X)	10.7 (2X)	3.14 (2X)	3.72 (2X)
3	0.90 (6X)	2.16 (4X)	21.4 (4X)	6.28 (4X)	7.44 (4X)
4	0.90 (6X)	3.24 (6X)	32.0 (6X)	9.42 (6X)	11.2 (6X)
Post-spike					
1	0.95 ± 0.03	0.54 ± 0.01	5.99 ± 0.50	1.35 ± 0.07	1.03 ± 0.06
2	0.96 ± 0.09	0.75 ± 0.05	10.1 ± 1.01	3.59 ± 0.21	1.46 ± 0.14
3	1.01 ± 0.03	1.58 ± 0.02	16.3 ± 0.30	7.31 ± 0.11	4.50 ± 0.24
4	1.01 ± 0.05	2.37 ± 0.06	22.6 ± 1.03	11.1 ± 0.23	4.57 ± 0.55
10 weeks					
1	0.73 ± 0.21	0.86 ± 0.07	5.64 ± 0.15	1.70 ± 0.02	1.67 ± 0.11
2	0.75 ± 0.04	1.11 ± 0.06	9.13 ± 0.36	3.09 ± 0.18	1.92 ± 0.28
3	0.75 ± 0.04	1.88 ± 0.01	13.7 ± 0.35	5.93 ± 0.04	4.23 ± 0.16
4	0.79 ± 0.03	2.76 ± 0.19	20.5 ± 0.9	9.73 ± 0.36	3.86 ± 0.04

(27 July), 4 (9 August), 6 (23 August), 8 (7 September) and 10 (26 September) weeks. The length of exposure was estimated as sufficient for metals to diffuse throughout the sediment (using Fick's law diffusion coefficient of 2 x 10^{-5} cm^2 s^{-1}).

Sampling of porewater and sediment

Porewaters and lake water just above the sediment-water interface were monitored throughout the experiment using *in situ* samplers or "porewater peepers" with 0.2 μm Gelman HT-100 polysulfone membrane, similar to those described by Hesslein (1976). The peepers consisted of Polyethylene sheets (1.3 cm thick) into which compartments (12 rows of two parallel compartments 1 cm apart; 1 cm vertical resolution; ~2.5 ml volume each) have been machined. The peeper tops were composed of Lexan plastic sheets (2 mm thick) held in place by stainless steel screws. Peepers were acid-washed with 10% HNO_3 and rinsed with deionized distilled water. Prior to installation, peepers were filled with deionized distilled water and deoxygenated by bubbling with nitrogen for 12 h in deionized distilled water. Peepers were inserted vertically into two randomly assigned trays in each treatment (n=2) and were sampled and replaced biweekly. Peepers extended 3 cm below and 9 cm above sediment-water interface. Porewaters were sampled by piercing the membrane of each compartment with an Eppendorf pipette fitted with an acid washed tip. The porewater was then transferred to an acid-washed polypropylene tube and acidified to 0.1% HNO_3. The two compartments in each row were combined into one sample to provide sufficient sample for analysis. Ambient pH of the porewater at different depths was measured in the field. Immediately upon removal of the peepers from the sediment a small volume of porewater (~100 μl) was extracted from each compartment and injected into a vial and its pH measured using a portable Orion pH meter (model 610) fitted with the rapid response Orion pHuture® sure-flow probe.

Sediments in the planter trays were sampled with a 5-cm internal diameter Plexiglass coring tube. One core per tray was collected for a total of 3 replicate sediment samples per treatment. Since the plant root system extended from the top to the bottom of the tray the whole core (~4-5 cm) was placed in a pre-washed centrifuge tube, filled with lake water and frozen within 2 h of collection. Prior to analysis sediments were thawed and centrifuged at 3,000 rpm to remove excess overlying lake water. The redox potentials of several samples were then measured using a Radiometer calomel half-cell (#K401) and platinum electrode (#P101). At the time of analysis sediments were oxidized with a Eh ranging from +248 to +400 mV. This Eh range is within that reported for sediments colonized by isoetids (~+300 to +600 mV; Tessenow and Baynes 1978).

Sampling of plants

Individual plant trays were divided into 5 quadrats to provide material for five sample times. One was chosen randomly at each sampling and all of the plants removed. Upon removal from the sediment, plants were placed in clean whirl-pak bags and filled with Roddy Lake water and transported to the field lab where they were stored at the current lake temperature until they were processed within 24 h. Plants were carefully cleaned in lake water using acid-washed forceps to remove debris and dead or dying root and shoot material; only healthy plant material was analyzed for metals. Normally plant material from a similar developmental stage is compared in order to control the effects of biological variability on metal uptake, but this is not possible with *E. septangulàre* which lacks a clear demarcation between life stages. Since many macrophyte species cause the precipitation of metals on their surface by the release of ligands and by oxygenating the area surrounding the root, steps were taken to distinguish between intracellular and extracellular metals. To remove extracellular metals, half of the plants (intact) were washed for 2 minutes in a Titanium (III) citrate (Ti (III)) solution adjusted to pH 7, then rinsed for 10 s in deionized distilled water(Hudson and Morel 1989). Titanium (III) citrate has been shown to reduce rapidly iron hydroxides with low cellular toxicity (Morel 1983). The untreated half of plants were rinsed for 10 s in deionized distilled water to provide a measure of total metal concentrations for comparisons between adsorbed and intracellular metals. The concentration of adsorbed metal was obtained by subtracting the total metal concentration in H_2O washed plants from the metal concentration in Ti (III) washed plants. After being washed, the plants were separated into roots and shoots and freeze-dried for metal analysis. The roots were separated from the rest of the plant just below stem (root-shoot transition zone) and the shoots just above. The stem was not included in the analysis since its morphology made it difficult to clean. Wet weight - dry weight ratios were determined on another set of plants (n=3 plants per replicate tray), by weighing separated roots and shoots, and drying them in an oven for 24 h at 60°C, after which they were placed into a dessicator to cool. Dry weights were then taken and % dry matter calculated by,

$$\% \text{ dry matter} = \frac{\text{dry wt. (g)}}{\text{fresh wt. (g)}} \times 100$$

Dry matter in plants consists of cellular components from the cell wall and within the cytosol (Salisbury and Ross 1992). Changes in % dry matter was used here as a broad assessment of the condition of the transplanted *E. septangulàre*.

Analysis of porewater, sediment and plant tissue

Metal concentrations (Cd and Fe) in acidified porewaters were measured by flame atomic absorption spectrophotometry (FAAS) and graphite furnace atomic absorption spectrophotometry (GFAAS) using a Varian GTA-95 (Varian Instruments, Georgetown, Ont.).

Sediment bound metals were partitioned into three operationally-defined fractions, easily-reducible (ER - associated with Mn oxides), reducible (R - associated with Mn and Fe oxides), and alkaline-extracted (ORG - bound to organic) using the simultaneous extraction method of Bendell Young et al. (1992) (Fig. 5.2). The ER extraction was expected to remove some of the most reactive amorphous Fe, but it is assumed that the majority of the Cd and metal mixture recovered from the ER fraction was associated with the oxides of Mn. The fraction of metals associated with only the Fe oxides (R-ER) was obtained by subtracting the ER fraction (containing only Mn-oxides) from the R fraction (containing both Fe- and Mn-oxides). Sediment-bound metals were also extracted with acid in an aqua regia digest (3:1 HCl and HNO_3, 80°C for 1 h) the results from which were defined as total acid-extractable metals (AE). Sediment metal concentrations (Cd, Cu, Zn, Pb, Ni, Fe and Mn) were measured by FAAS and GFAAS using a Varian GTA-95 (Varian Instruments, Georgetown, Ont.) and standards with appropriate matrices of extraction solutions. All metal concentrations in sediment were expressed on a dry weight basis. A separate aliquot of sediment was used for a wet/dry weight comparison (dried at 60°C for 24 h). The partitioning of metals in sediments are reported for background, post-spike and only 2, 8 and 10 week sediments. Precision of the extraction technique, measured by subsampling one core from each treatment during each extraction, was less than 12% (CV), with few exceptions, for all metal fractions.

Root and shoot tissues were digested with conc. HNO_3, oxidized with 30% H_2O_2 and analyzed for metals using FAAS and GFAAS using a Varian GTA-95 or polarized Zeeman Z-8200 with Zeeman background correction (Hitachi Scientific Instruments,

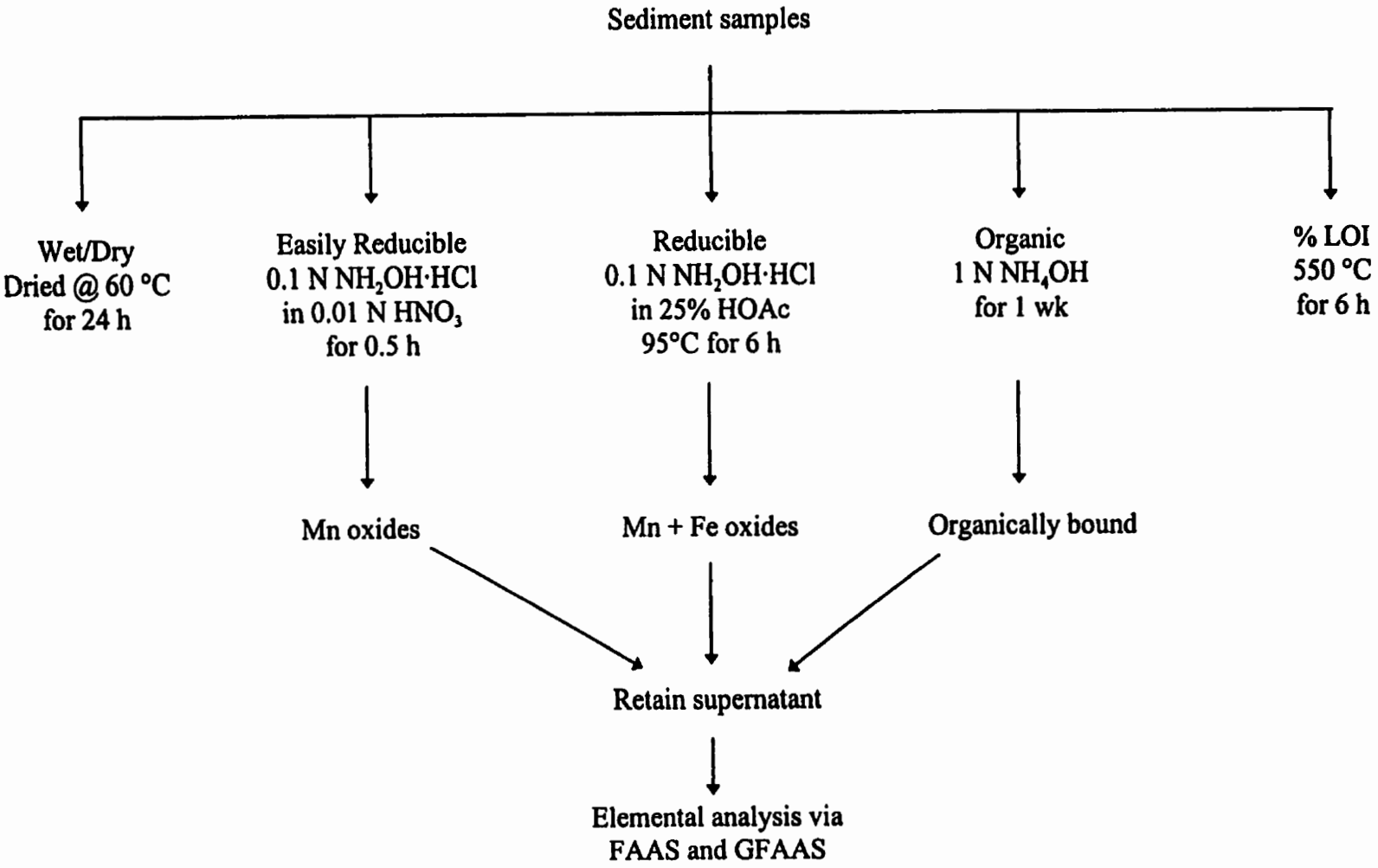


Figure 5.2. Simultaneous extraction procedure. Redrawn from Bendell Young et al. (1991).

Canada). Duplicate samples of National Bureau of Standards reference material #1572 Citrus leaves were analyzed with every set of plant samples. Measured metal concentrations (means ±SE, n=3-4) were within certified ranges for each metal (shown in parentheses): Cd 0.05 ± 0.04 μg g^{-1} (0.03 ± 0.01 μg g^{-1}), Cu 17.2 ± 1.3 μg g^{-1}(16.5 ± 1.0), Zn 28.3 ± 1.1 μg g^{-1} (29 ± 2 μg g^{-1}), Pb 12.8 ● 1.4 μg g^{-1} (13.3 ± 2.4 μg g^{-1}), and Ni 0.83 ± 0.35 μg g^{-1}(0.6 ± 0.3 μg g^{-1}). The Cd concentrations measured in the reference materials were slightly higher and more variable than the certified ranges, however the certified concentrations were also considerably lower than the concentration range used to measure metals in the plants exposed to the spiked sediment. Variability in plant metal concentrations among replicate trays was high (range of 4-60% CV for shoots and roots), but was similar to that for indigenous plant material (*Nuphar variegatum*) reported by others (Campbell et al. 1985).

All glassware used in the metal analyses was washed using conc. HNO_3 (reagent grade) and rinsed at least 3 times with deionized distilled water.

Statistical methods

Data were tested for normality using the Shapiro-Wilk statistic, a ratio between the best estimator of the variance and the corrected sum of squares estimator of the variance, and log transformed when required. Statistically significant differences in metal concentrations in sediment fractions and plant parts among treatments for each of the sample weeks were determined using SAS version 6.08 ANOVA with treatment as a factor (SAS Institute Inc., 1989). Differences in metal concentrations in sediment fractions and plant parts over the whole exposure period were determined using repeated measures ANOVA with treatment and time as factors and tested against the replicate tray error within treatment (SAS Institute Inc., 1989). Correlations between sediment fractions were determined using SAS version 6.08 correlation analysis, with Pearson correlation coefficients.

5.4 Results

Post-spike sediment metal concentrations

The measured acid-extracted AE metal concentrations were close to nominal for Cd and Pb (Table 5.2). Zn concentrations were ~80% of nominal in treatments 3 and 4 and Cu concentrations were ~72% of nominal in all treatments. Nickel concentrations in spiked sediments were the least similar to nominal values, with actual concentrations only 47% of nominal.

Distribution of Cd in the sediment

The distribution of Cd and the other metals in background samples, post-spike sediment and after 2, 8 and 10 weeks is shown in Figs. 5.3 and 5.4. In background sediment from Roddy Lake, the largest proportion of Cd was associated with the organic fraction (~80%) followed by the ER (~60%) and R-ER (~25%) fractions (Fig. 5.3a,5.4a). Note that these fractions are not mutually exclusive and when the concentrations of Cd in each fraction is added together the total amount may exceed that in the AE fraction. The amount of Cd in the AE fraction (0.15 $\mu g\ g^{-1}$ dry wt.) was slightly higher than that predicted using the regression equation for Cd in littoral sediments and %LOI (0.085 $\mu g\ g^{-1}$ dry wt.) developed by Stephenson and Mackie (1988), but was within their range found for central Ontario littoral sediments (0.01 to 2.51 $\mu g\ g^{-1}$ dry wt.).

Treatment related differences in the partitioning of Cd in all of the sediment fractions were observed in post-spike sediments and after 2, and 8 weeks. Repeated measures ANOVA on [Cd] data from 2, 8 and 10 weeks found significant treatment effects for Cd in the ER, R-ER and ORG fractions, and for the proportion of Cd associated with ER (% of AE) and R-ER (% of AE)) fractions relative the AE fraction(Table 5.3). A significant interaction was found for treatment x week for Cd in the AE and ER fractions (Table 5.3). Specific differences among treatments are described below.

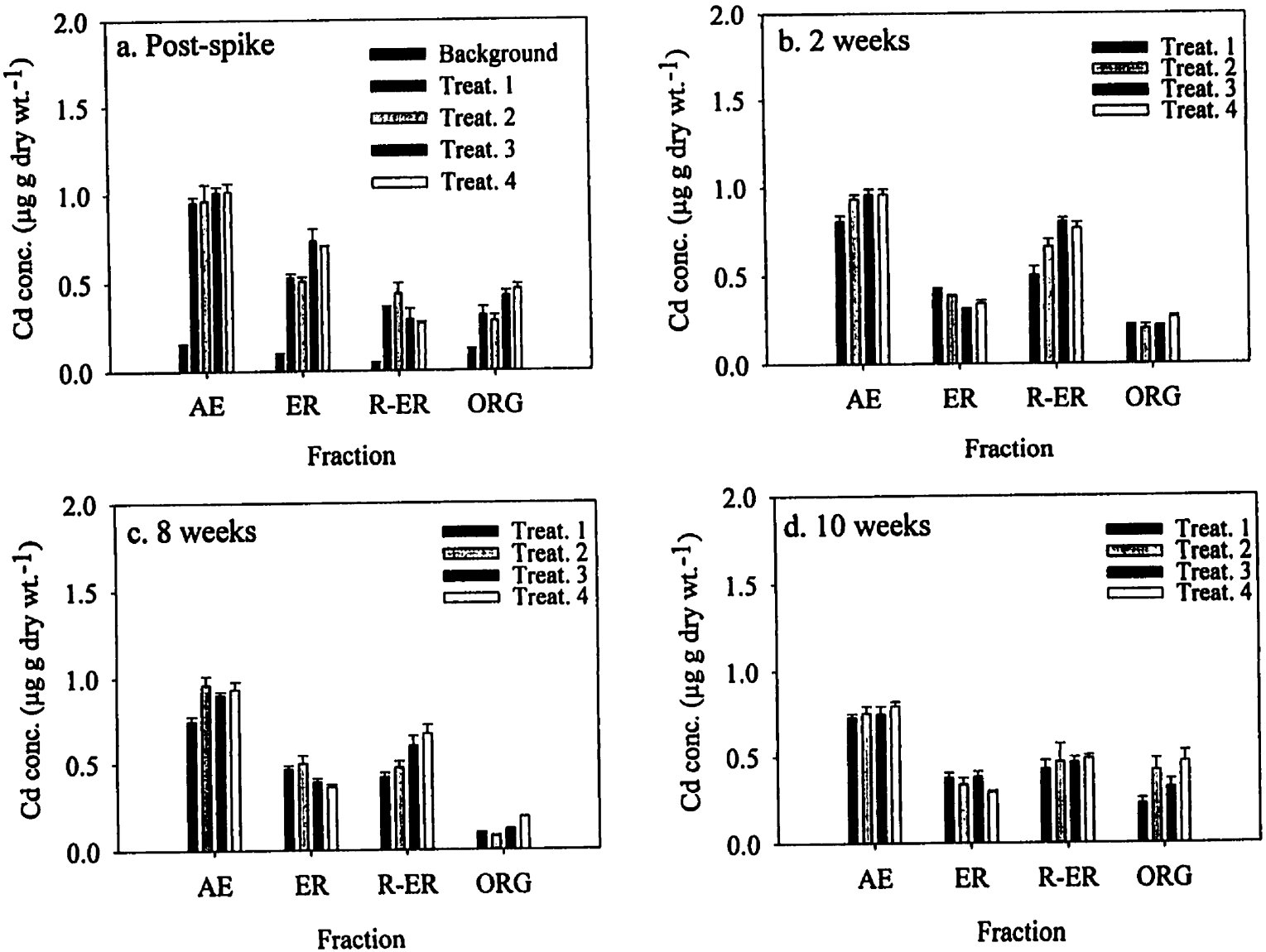


Figure 5.3. Cadmium concentration (µg g^{-1}) in each of the geochemical fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).

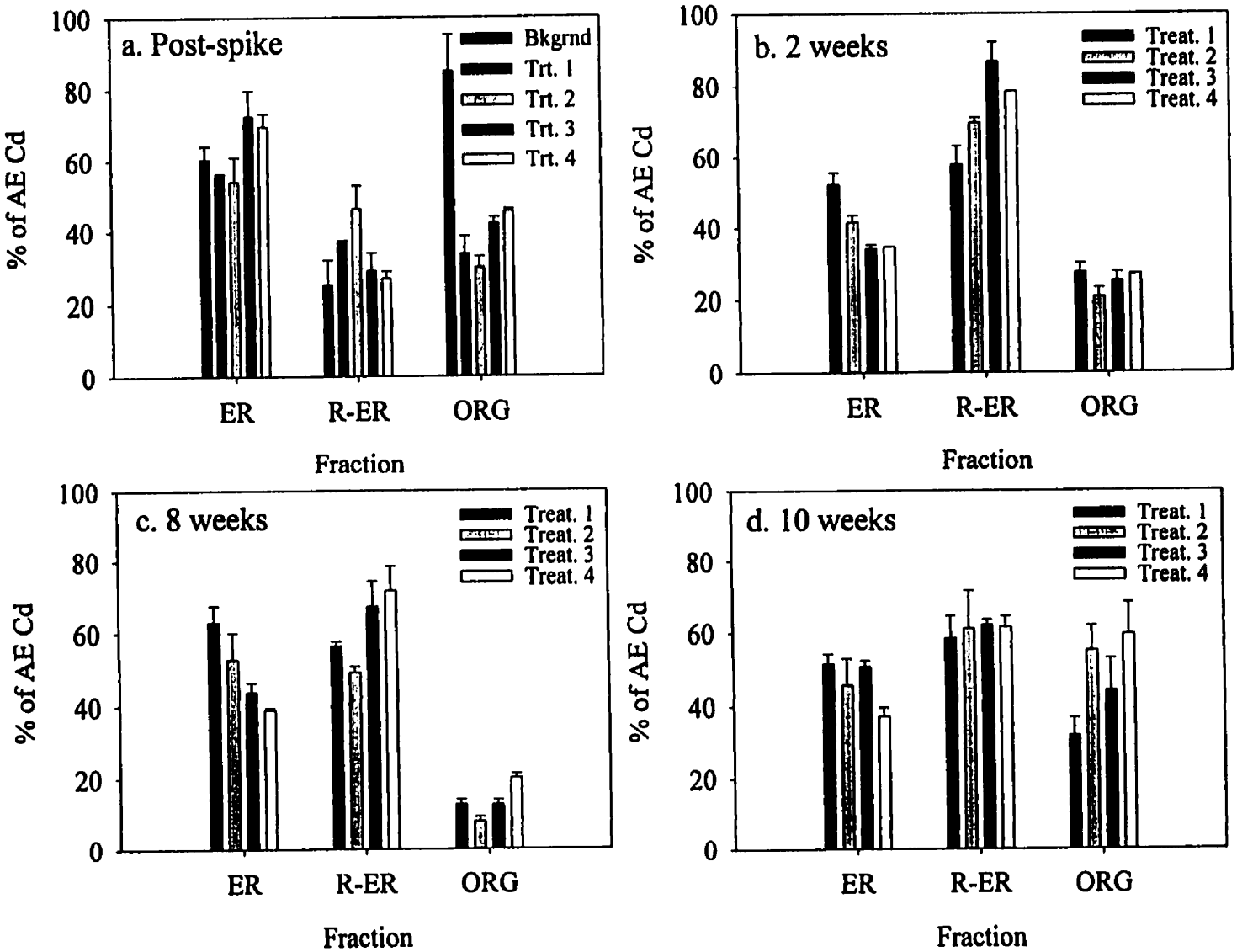


Figure 5.4. Proportion of Cd associated with the ER, R-ER and ORG fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).

Table 5.3. Repeated measures ANOVA table for differences in the partitioning of Cd into geochemical fractions among treatments over 2, 8 and 10 weeks.

Geochemical Fraction	Factor	df	F	*P* value
AE	Treatment	3	4.85	**0.03**
	Tray(Treatment)	8	4.34	0.006
	Week	2	51.4	<0.001
	Treatment x Week	6	3.43	0.02
ER	Treatment	3	5.19	**0.03**
	Tray(Treatment)	8	2.52	0.05
	Week	2	19.6	<0.001
	Treatment x Week	6	3.76	0.02
R-ER	Treatment	3	6.06	**0.02**
	Tray(Treatment)	8	2.27	0.08
	Week	2	30.5	<0.001
	Treatment x Week	6	2.50	0.07
ORG	Treatment	3	8.67	**0.007**
	Tray(Treatment)	8	0.85	0.57
	Week	2	51.2	<0.001
	Treatment x Week	6	2.56	0.06
ER % of AE	Treatment	3	7.71	**0.01**
	Tray(Treatment)	8	3.11	0.03
	Week	2	10.32	0.001
	Treatment x Week	6	3.21	0.03
R-ER % of AE	Treatment	3	2.68	**0.012**
	Tray(Treatment)	8	2.69	0.04
	Week	2	13.3	<0.001
	Treatment x Week	6	1.97	0.13
ORG % of AE	Treatment	3	3.33	0.08
	Tray(Treatment)	8	1.28	0.32
	Week	2	66.1	<0.001
	Treatment x Week	6	2.96	0.04

Acid-extractable Cd

From the time the trays were introduced into the lake until the end of the exposure period there was an approximately 25% loss of Cd from all of the treatments to the overlying water. Post-spike sediments, measured one day after the metal spike, revealed

no statistically significant differences in [Cd(AE)] among treatments (Fig. 5.3a), but after 2 and 8 weeks, the [Cd(AE)] was significantly lower in treatment 1 relative to that in treatments 2-4 ($P<0.05$) (Fig. 5.3b and c). By 10 weeks, differences in [Cd(AE)] among treatments were not statistically significant (Fig. 5.3d).

Easily-reducible Cd

The ER fraction was a significant source of Cd in post-spike sediments (Fig. 5.3a). The proportion of Cd associated with the ER fraction was higher in post-spike samples (~55-75%) than in 10 week samples (~35-55%) (Fig. 5.4a-d). The metal mixture appeared to have an influence on the partitioning of Cd in the ER fraction in post-spike sediments, whereby treatment 1 and 2 [Cd(ER)] were significantly lower than those in treatment 3 and 4 ($F=9.6$, $P<0.01$). After 2 and 8 weeks, the proportion of Cd in the ER fraction in treatments 3 and 4 decreased to levels significantly lower than those in treatments 1 and 2 ($P<0.05$) (Fig. 5.4b and c). There were no significant differences among treatments in the ER fraction after 10 weeks (Fig. 5.3d).

Reducible Cd (R-ER)

There were no significant differences in the partitioning of Cd in the R-ER fraction among treatments in post-spike sediments, although [Cd(R-ER)] appeared somewhat lower in treatments 3 and 4 relative to treatments 1 and 2 (Fig. 5.3a). After 2 and 8 weeks the [Cd(R-ER)] significantly increased in treatments 3 and 4 from post-spike levels corresponding to a shift in Cd from the ER fraction. At this time [Cd(R-ER)] were significantly lower in treatment 1 and 2 relative to treatments 3 and 4 ($P<0.05$). Similar statistically significant differences were found for the proportion of Cd associated with the R-ER fraction (Fig. 5.4b-d). A significant inverse correlation between treatment [Cd(R-ER)] and treatment [Cd(ER)] was found in 2, 8 and 10 week samples (Pearson $r=-0.439$, $P=0.007$).

Organic Cd

The ORG fraction was not a significant sink for Cd during the 10 week exposure except in post-spike sediments and after 10 weeks in some treatments (Fig. 5.3 and 5.4).

Approximately 30-45% of the Cd was associated with the ORG fraction in post-spike sediments which was slightly higher than that associated with the R-ER fraction (Fig. 5.3a). The [Cd(ORG)] were significantly higher in treatment 4 than in treatment 1 and 2 and in treatment 3 than in treatment 2 ($F= 6.2$, $P=0.02$). This same trend was found after 8 weeks exposure ($F=30$, $P=0.0001$) (Fig. 5.3c).

Distribution of other metals in the sediment

As expected, the concentration of Cu, Zn and Pb increased with treatment level in all sediment fractions (Appendix 10.1-10.3). Ni concentrations in spiked sediment did not reflect treatment levels and therefore its distribution among sediment fractions will not be discussed. The proportion of metals associated with the different geochemical fractions varied for each metal and over the exposure period (Appendix 10.6-10.8). The largest proportion of Cu was initially associated with the ORG fraction (~80%), but then slowly declined as more Cu shifted onto the R-ER fraction. Initially, all of the added Zn (treatments 2-4) was associated with the ER fraction. After 2 and 8 weeks, the Zn was distributed more or less equally between the ER and R-ER fractions with the ER fraction having a larger proportion of the Zn at the highest concentrations of the other metals. At the highest treatment concentrations, Pb was initially associated with the R-ER fraction and ER fraction, but after 2 and 8 weeks shifted predominantly into the R-ER fraction with lesser amounts in the ER fraction.

The partitioning of Fe among geochemical phases in spiked sediment over the exposure period was similar to background sediments (Appendix 10.4). Roughly 25% of the Fe was associated with the R-ER fraction and none was associated with the ER and ORG fractions (Appendix 10.9). As expected, the largest proportion of Mn was associated with the ER fraction (50%) and the next largest proportion with the R-ER fraction (~25%) (Appendix 10.10). After 10 weeks a small amount of Mn (~15%) was associated with the ORG fraction.

Cd in porewater

Variability among the replicate peepers for each treatment was high (mean coefficient of variation (CV) ~60%). This may have been caused by inadvertent

contamination of porewater during sampling of the compartments. Small metal-laden particles attached to the outside of the peeper membrane could have been taken along with the water sample when the pipette tip pierced the membrane. Porewater Cd profiles in the sediment over time are shown in Appendix 10.11. Porewater [Cd] were highest near the bottom of the trays and decreased exponentially upwards in the sediment and were near background above the sediment-water interface. At no time during the exposure were significant differences found in porewater [Cd] among treatments. Porewater [Fe] showed a similar exponential decrease from the deepest layer towards the surface, except elevated [Fe] were maintained above the sediment-water interface (Appendix 10.12). An overall decrease of [Fe] in porewater was observed from 4 to 10 weeks.

The porewater pH tended to be lower in the sediment compared to overlying water ranging from ~6.2 to 6.7 below the sediment to ~6.7 to 7.5 above the sediment-water interface at 4 weeks (Appendix 10.13). The range in pH in porewater and surface water was relatively narrow from 6 to 10 weeks compared to 4 weeks. The time period of 6 to 10 weeks extended from the end of August to the middle of October. At this time of the year photosynthesis by benthic algae and *E. septangulàre* would be expected to be lower than at 4 weeks (early August) resulting in reduced consumption of CO_2, which normally drives up water column pH during the day. At no time during the exposure were there significant differences in pH among treatments.

Changes in plant % dry matter

The proportion of dry matter in the roots and shoots of transplanted *E. septangulàre* in treatment 4 after 10 weeks *in situ* was significantly higher than that measured in specimens from Lake 104 (Table 5.4). The % dry matter in treatment 4 plants was also significantly higher in the roots of treatment 1 plants and in the shoots of plants from all of the other treatments. Overall, shoot % dry matter increased from the beginning of the exposure to 10 weeks in all treatments and in Lake 104 specimens.

Table 5..4. Changes in % dry matter in *E. septangulàre* over the 10 week exposure period compared to plants freshly collected from Lake 104. Values are means (±SE, n=3, except Lake 104 where n=1 pooled sample).

		% Dry matter				
Part	Week	Lake 104	Treat. 1	Treat. 2	Treat. 3	Treat.4
Root	0	5.47 ± 0.71				
	2	na	9.06 ± 3.87	5.78 ± 1.27	8.73 ± 1.06	7.24 ± 0.46
	10	4.47 ± 0.89	5.22 ± 0.75	6.91 ± 1.07	6.66 ± 1.06	7.99 ± 0.98*
Shoot	0	7.33 ± 0.83				
	2	na	11.79 ± 0.78	13.0 ± 3.0	17.0 ± 0.9	11.3 ± 0.5
	10	14.9 ± 0.6	14.7 ± 2.2	18.4 ± 3.2	16.3 ± 2.2	24.9 ● 4.3**

Root * - Treatment 4 is significantly different from treatment 1 and Lake 104 plants ($P<0.05$).
Shoot ** - Treatment 4 is significantly different from all other treatments and Lake 104 plants ($P<0.01$).

Adsorbed Cd in H_2O washed plants - External concentrations

The Ti (III) wash removed a significant amount of metal adsorbed on the plants. Adsorbed Cd on *E. septangulàre* accounted for 57% and 35% of the total Cd extracted from the roots and shoots, respectively (Fig 5.5). The difference in [Cd] in Ti (III) washed and H_2O washed plants was highly significant in the roots on all sample days (2 - 10 weeks) and in the shoots from 4-8 weeks ($P<0.05$). No treatment related differences in the amount of [Cd(adsorbed)] on either the roots and shoots were observed.

Cd accumulation in Ti (III) washed plants - Internal concentrations

Cadmium accumulated in the roots and shoots of *E. septangulàre* over the 10 week exposure period as shown by the analysis of Ti (III) washed plants (Fig. 5.6a and b). Cd concentrations were approximately 10 X higher in the roots than in the shoots. Cd

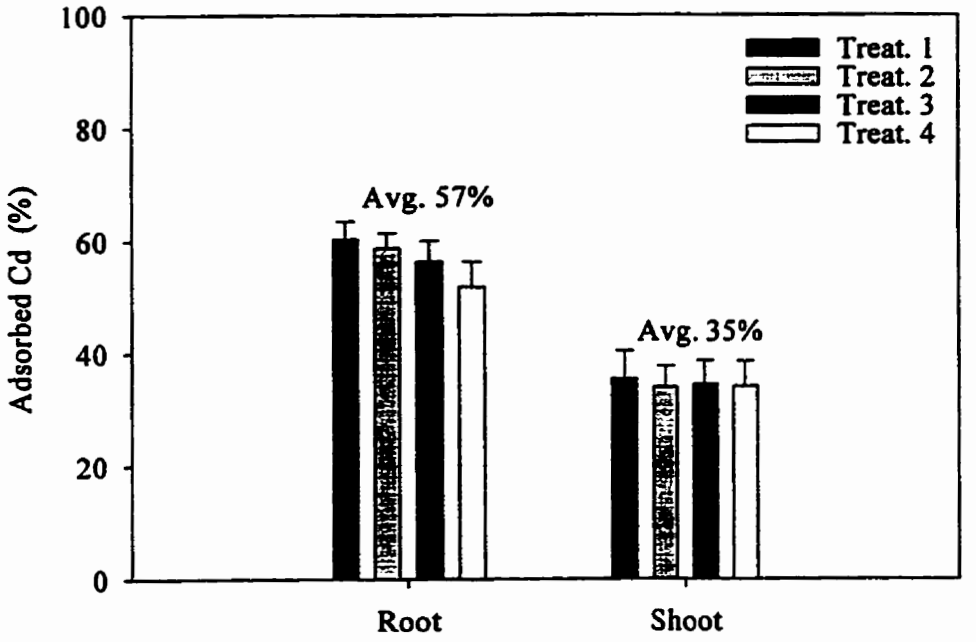


Figure 5.5. Proportion of total Cd adsorbed on roots and shoots of *E. septangulàre*. Values are means (n=3) of [Cd(Ti (III) washed)]/[Cd(H_2O washed)] for plants collected over the exposure period.

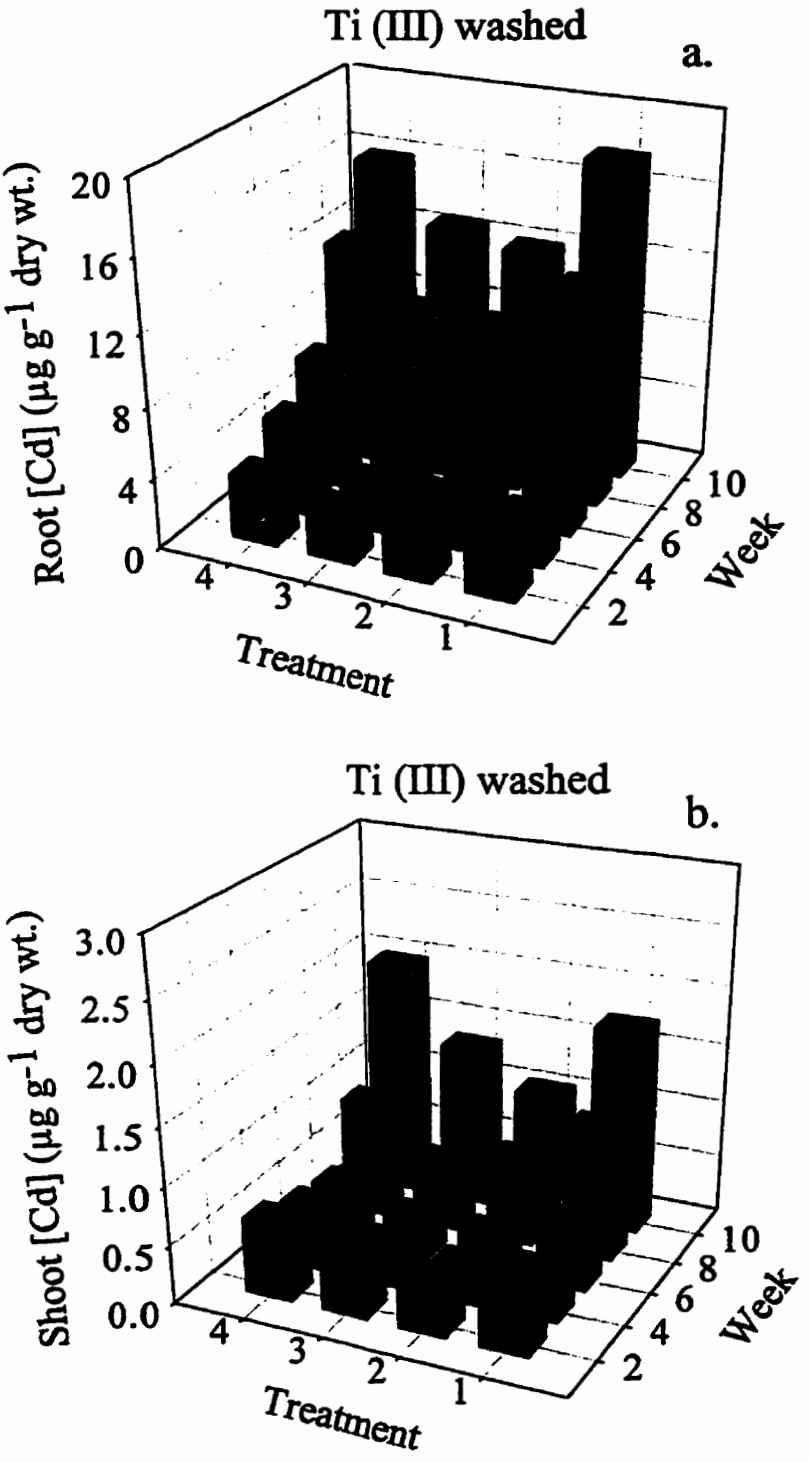


Figure 5.6. Accumulation of Cd in $TiCl_3$ washed *E. septangulàre* over the 10 week exposure period. a. Roots. b. Shoots. Values are means (n=3).

uptake in the roots was initially slow during the first 4 weeks of exposure and was followed by a steady increase that may have continued after 10 weeks, except in treatment 4 which increased steadily from the beginning of the exposure. Root [Cd] were significantly different from background concentration in Lake 104 plants after 6 weeks exposure (F=10.74, P=0.003). In the shoots, there was an initial increase in Cd accumulation followed by a slight decrease from 4 to 6 weeks and then steady increase thereafter until 10 weeks. Shoot [Cd] in all treatments were significantly different from background after two weeks exposure (F=10.5, P=0.003). As with the roots, shoot [Cd] did not show signs of reaching steady state after 10 weeks *in situ*.

There were no statistically significant effects of treatment on root [Cd] over the entire exposure period (Table 5.5). On the other hand, the repeated measures test over the entire exposure period indicated significantly higher shoot [Cd] in treatments 1 and 4 compared to those in treatments 2 and 3. Both root and shoot [Cd] increased significantly over time.

5.5 Discussion

Effect of the metal mixture on the distribution of Cd in spiked sediments

The metal mixture added with Cd to uncontaminated lake sediments influenced the distribution of Cd among geochemical fractions with different binding strengths. After initial sorption onto the ER fraction, the added Cd shifted during the course of the experiment onto the R-ER and ORG fractions at high concentrations of other metals. Literature on Cd binding to sediment reports that the affinity of Cd for natural substrates follows the order Mn > Fe (amorphous) > chlorite > Fe (crystalline) = illite = humics > kaolinite > silica (Laxen 1983). There are examples of spiked Cd rapidly partitioning onto Mn-oxides. For example, Cd applied to estuarine sediments quickly partitioned onto an easily reducible (Mn oxyhydroxide) fraction and repartitioned onto an organic and sulfide fraction with time (Rule and Alden 1992; Rule and Alden 1996a). Cadmium added experimentally to the epilimnion of Lake 382 during the ice-free season associated with the Mn-oxyhydroxides and organic fraction (same extraction scheme used here) in depositional zones and with Fe-oxyhydroxides in shallow sandy sediments (Stephenson

Table 5.5. Repeated measures ANOVA table for differences in root and shoot Cd concentrations among treatments over the exposure entire exposure period (2-10 weeks). Significant *P* values are shown in bold.

		Root			Shoot		
	Factor	df	F	*P* value	df	F	*P* value
Ti (III) washed plants	Treatment	3	0.99	0.44	3	6.79	**0.01**
	Tray(Treatment)	8	1.36	0.26	8	1.30	0.28
	Week	4	52.1	**<0.001**	4	48.4	**<0.001**
	Treatment x Week	12	0.88	0.57	12	0.79	0.66
H_2O washed plants	Treatment	3	1.70	0.24	3	3.58	0.05
	Tray(Treatment)	8	2.09	0.07	8	1.27	0.31
	Week	4	66.4	**<0.001**	4	34.8	**<0.001**
	Treatment x Week	12	0.90	0.56	12	0.58	0.84

et al. 1996). The authors suggested that over time diagenetic processes may result in the transfer of Cd from Mn-oxides to more stable sites such as Fe oxyhydroxides and sulfides. Tessier et al. (1993) concluded that the majority of the Cd bound to natural littoral sediments in 38 Ontario and Quebec lakes receiving trace metals from atmospheric deposition was associated with organic matter. The analysis of background sediments from Roddy Bay indicated that virtually all of the Cd was associated with the organic fraction.

The repartitioning of Cd onto the R-ER fraction at the highest concentrations of the metal mixture may have been caused by competition for binding sites on the preferred Mn-oxide fraction. The relative affinities (calculated as K_d = concentration of sorbed metal/ concentration of dissolved metal) of the different metals for a synthetic Mn oxide substrate (Buserite) followed the order Pb > Zn > Cd ≈ Ni (Balistrieri and Murray 1986). The affinity order for freshly precipitated Fe oxyhydroxides is reported to follow the order Pb > Cu > Zn > Ni > Cd (Kinniburgh et al. 1976). Based on the overall lower affinity of Cd for Mn oxides compared to the other metals it is possible that the other metals bound to the Mn oxides and forced Cd onto the other available substrates including Fe-oxides and organic material. The increasing proportion of Zn in the ER fraction relative to AE Zn with increasing treatment level at 2, 8 and 10 weeks suggests that Zn may have been involved in the re-partitioning of Cd onto the R-ER fraction.

Rule and Alden (1996a) found Cu to have an effect on the distribution Cd among geochemical fractions in spiked anaerobic estuarine sediments. As more Cu was added, the amount of Cd extracted from fractions representing an exchangeable phase and Mn-oxyhydroxide increased. The authors suggested that the Cd shifted from an organic-sulfide fraction, where it normally was found in estuarine sediments, in response to the increasing Cu concentrations (Rule and Alden 1996a).

Apart from competition from other metals, an alternative explanation for the differences in sorption among treatments is that Cd was co-precipitated with the other metals onto Fe-oxides at the highest treatment levels after solubility limits were exceeded. This alternative does not appear likely. The solubility limits for the Fe-oxide phase were likely not exceeded due to the low metal concentrations used in this study and at the time of highest metal concentrations (post-spike) when precipitation could have

occurred only small amounts of Cd associated with Fe-oxides. The shifts in Cd from the ER fraction to the R-ER and ORG fraction also does not support co-precipitation since metals are usually bound within the matrices of the precipitated Fe-oxide complex and are less exchangeable after they have precipitated. Furthermore, co-precipitation and adsorption experiments using hydrous ferric oxide have shown that the difference between the two removal processes are virtually indistinguishable for Cd, Cu, Pb and Ni under conditions typical of natural freshwaters (Laxen 1985). Co-precipitation of Cd was found to be highly dependent on alkalinity, which Laxen (1985) suggests is the result of competition from Ca and Mg ions. Consequently, at the highest concentrations of the other metals competition for binding sites on precipitating Fe-oxide from the other metals might have been expected which should have reduced the amount of Cd in those treatments and not caused the observed increase. Conditional adsorption constants for Cd, Pb and Cu measured on precipitating hydrous ferric oxide followed the order Pb > Cu > Cd (Laxen 1984).

After 10 weeks *in situ*, the effect of the metal mixture on the partitioning of Cd among geochemical fractions became less evident and overall metal concentrations decreased in all fractions, except the organic fraction. Over time it is not surprising for the spiked sediment to become more stable (see uniform pH profiles, Appendix II) which would lead to a more equitable distribution of Cd among sediment fractions, i.e., Mn- and Fe-oxides. The role of *E. septangulàre* in the distribution of Cd after 10 weeks is discussed below.

Effect of transplantation and metal exposure on plant % dry matter

Transplanting *E. septangulàre* from one lake to another and exposure to metals did not appear to have a deleterious effect on plant "condition", since % dry matter in the roots and shoots of transplanted *E. septangulàre* was the same as or higher than that in Lake 104 plants. The significant increase in % dry matter in treatment 4 roots and shoots relative to the other treatments and Lake 104 plants indicates that transplanting and/or exposure to the metals may have influenced the condition of *E. septangulàre*. The fact that the increases were only observed in treatments 2-4, suggests that the exposure to the metal mixture may have led to the increase in % dry matter, possibly through the

availability of essential metals (Cu, Zn, Ni) or by the fertilization effect of the $Pb(NO_3)_2$ addition. Adverse effects from exposure to Cd and the metal mixture were unlikely. The toxicity threshold (EC_{50}) for *E. septangulàre* is not known, but has been measured at 130-1200 μg Cd g^{-1} dry weight for *Lemna trisulca* (Huebert 1992), which is considerably higher than the highest root [Cd] (<50 μg g^{-1} dry weight) observed after 10 weeks *in situ* exposure in the present experiment.

Despite the positive changes in % dry matter, the plants may have experienced some form of transplant stress based on metal accumulation patterns in the roots and shoots. Metal uptake in the roots and shoots showed an initial lag period of approximately 4 weeks which was followed by a more steady increase in metal accumulation. This period of steady increase may have been a signal that the plant had become established and was physiologically stable, although it is impossible to be sure since there was no transplant control, i.e., tray of plants in background sediment. Further study of transplant stress in *E. septangulare* would be required in future studies using this species.

Effect of the metal mixture on the accumulation of Cd by plants

The consequence of the repartitioning of Cd from the Mn-oxide or "easily reducible" to the Fe-oxide or "reducible-easily-reducible" fraction is that the Cd may have actually become less bioavailable to aquatic organisms. However, Cd accumulation in the roots and shoots of *E. septangulàre* among treatments was not consistent with this hypothesis. Despite the apparent increase in the amount of Cd associated with the less exchangeable fractions, treatment 4 root and shoot [Cd] were similar to those measured in treatment 1 and higher than those in treatments 2 and 3. The higher shoot [Cd] in plants from treatment 1 relative to treatments 2 and 3 corresponds to the higher [Cd] in treatment 1 associated with the most easily reducible or "bioavailable" fraction. The Cu contents of the water lily *Nuphar variegatum* collected downstream from a Cu/Zn mining and smelting complex in Northwestern Quebec, was found to be significantly correlated to Cu concentrations in fractions most easily extracted from sediment (Campbell et al. (1985). No other studies have examined the relationship between distribution of Cd among sediment fractions and its affect on uptake by aquatic macrophytes. In laboratory

experiment using mussels, Rule and Alden (1996b) found that [Cd] in the blue mussel *Mytilus edulis* exposed to Cd and Cu in spiked sediments were significantly correlated to [Cd] associated with an easily reducible fraction (3^3 factorial, 14 d, sediment concentration range - Cd 0 - 5 μg g^{-1} dry wt., Cu 0 - 25 μg g^{-1} dry wt.). The higher shoot [Cd] in treatment 4 were surprising since significantly more Cd in treatment 4 sediments partitioned into the less bioavailable R-ER fraction relative to treatments 2 and 3. In treatment 4 with the highest concentrations of the other metals, interactions with other metals may have enhanced uptake. Huebert and Shay (1992) found that at low external [Zn] in *Lemna trisulca* (axenic culture, aqueous exposure; 0.08-6.12 μM) Cd uptake was either not affected or decreased, but at high levels of Zn (12.2 μM) Cd uptake was enhanced. Accumulation of elements in plants is thought to be mediated by transport proteins in the cell membrane that are element specific (Salisbury and Ross 1992). Absorption of some elements may take place inadvertently via ion-pumps for other essential cations (i.e. Cd in a Ca-pump) or by passive diffusion through leaky membranes of compromised plants (Salisbury and Ross 1993). The mechanism by which one metal could enhance the uptake of another has not been described. Alternatively, at the high exposure concentrations in treatment 4 it's possible that the cell membranes became leaky, thus allowing more metal to be adsorbed relative to treatments 2 and 3. However, if this were true then a greater portion of Cd would have been removed by the Ti (III) wash, since it would have removed some of the internal Cd in addition to the adsorbed Cd, which did not happen.

Effect of the metal mixture on adsorbed Cd

Macrophytes that possess the isoetoid growth form are particularly effective at oxygenating their substratum. The release of oxygen from the roots of isoetids and other macrophytes is thought to be an adaptive response to living in areas with low dissolved inorganic carbon, since the oxygen stimulates CO_2-generating decomposition processes in the sediment (Wium-Andersen and Andersen, 1972). But the oxygen released into the sediment can also cause the precipitation of Fe and Mn as oxides which can also result in the removal of other trace metals. The Ti (III) reagent removed a larger proportion of adsorbed metal from the roots compared to the shoots indicating that more metals were

precipitated on the roots, consistent with the leakage of oxygen from the roots. Laboratory experiments by Wium-Andersen (1971) found that oxygen liberation from the roots of *Lobelia dortmanna* was 3-5 times greater than that liberated from the leaves. The precipitation of potentially toxic trace metals as Fe- and Mn-oxides is probably a useful by-product of the oxygen release and not necessarily a response of the plant to exposure to high trace metal concentrations. Evidence for this was provided in the present experiment by the lack of an interaction between metal exposure concentration (treatment level) and wash in the present study. Therefore, it is unlikely that the oxygenation of the substratum played a significant role in the determining the treatment related patterns of metal uptake. On the other hand, it may have influenced the partitioning of Cd among sediment fractions. After 10 weeks there were no significant differences in the partitioning of Cd among the ER and R-ER fractions. At this time, one fourth of the plants had been removed from the trays and as a consequence the sediments would have been less oxygenated reducing the formation of Mn- and Fe-oxides. The oxidizing effect of isoetid roots on the sediment is a local phenomenon along the length of the root (Smits et al. 1990) and in very sandy sediments, similar to those in this study, extends outward less than 0.1 m^2, which makes the above suggestion possible (Wium-Andersen and Andersen 1972). Furthermore, photosynthesis in *E. septangulàre* at this time of year would have been relatively lower compared to other sample periods, thus reducing the release of oxygen to acquire photosynthetic inorganic CO_2.

Conclusions

A metal mixture of Cu, Zn, Pb and Ni had a significant effect on the distribution of Cd among geochemical fractions in spiked littoral sediments. At the highest concentrations of the metal mixture, some Cd shifted from the Mn-oxide fraction to the Fe-oxide and organic fractions where metals are considered to be less bioavailable. Despite the lower bioavailability of the Cd in treatment 4 sediments, uptake of Cd in both the shoot and roots was enhanced relative to treatments with lower concentrations of the metal mixture, possibly due to synergistic effects of the other metals. We observed that plant [Cd] did not reach steady-state during the exposure period. The absorption patterns suggest that the first four weeks was required by the plants to acclimatize to their new

environment. It is recommended that in future uptake studies using transplanted *E. septangulàre* the exposure period be longer than 10 weeks to allow for recovery from the stress of transplantation.

CHAPTER 6. GENERAL DISCUSSION

Under the Constitution Act (1982), the federal government has legislative responsibility for Canada's fisheries (Department of Fisheries and Oceans 1986). The Minister of Fisheries and Oceans has been assigned responsibility for sea coast and inland fisheries, marine science and administrations of the Fisheries Act. This responsibility includes comprehensive powers to protect fish (i.e., applies to fish, shellfish, crustaceans, marine animals and plants) and fish habitat (i.e., those parts of the environment depended on directly and indirectly for life processes) from the discharge of deleterious substances such as toxic metals. Protection of fish and fish habitat can be achieved through the development of regulations (e.g., MMLERS, water quality guidelines) and monitoring tools that evaluate the effectiveness of the regulations. For example, biomonitoring organisms have been shown to be useful tools for the assessment of industry's efforts in meeting environmental regulations.

A variety of organisms have been examined as biomonitoring tools for metals including fish, benthic invertebrates, macrophytes and algae. The effectiveness of any one organism as a biomonitor depends on our understanding of the organism and their interactions with metals, and most importantly their limitations as a biomonitor. After completing Cycle I of the Pulp and Paper Environmental Effects Monitoring Program (EEM), regulators concluded that adult fish surveys alone could not provide the information needed to determine if an "impact" had occurred. Regulators have recently begun to look at molluscs which as sedentary organisms could provide a site specific assessment of impacts (Stewart 1997). The ability to cage molluscs offered advantages to perform more controlled studies of contamination impacts.

In the case of molluscs as biomonitoring tools, there are still many questions that need to be answered. The most critical questions lie with our understanding of the relationship between tissue-residue metal levels and effects at the cellular, physiological, and community levels. Unfortunately, questions dealing with changes at the population and community levels usually require longer periods of study accompanied by the need

for secure levels of funding, a phenomenon that is becoming increasing rare throughout Canada.

Identifying the most active or potent agents of toxicity in the environment is exceedingly difficult. Furthermore, the dearth of scientific literature on mixtures and the lack of consensus on the behavior of mixtures has made it impossible to predict the potential risk of multiple metal exposures at this time (Luoma and Carter 1991). The results presented here indicate that in less than 80 d a metal mixture influenced the distribution of Cd in water and sediment. Furthermore, the ELA where the *in situ* experiments were conducted is comparably pristine and does not carry a significant contaminant burden. It would not be surprising that in a more contaminated system at or near saturation that competitive interactions among the metals may be more intense. For example, in the limnocorral experiment if the sediments had been near saturation, the removal rates of Cd from the water column may have been slower that those observed. The resulting effects of the metal mixture on *P. grandis* and *E. septangulare* were less clear and require further investigation. The question of the effect of high metal concentrations on filtration rates in bivalves is significant and has serious implications for the use of bivalves as monitoring organisms and should have top priority for future research. The transplantation and exposure of *E. septangulare* provided important information for further study of metal uptake in this species. As with mussels, transplant stress is a factor in aquatic plants and should be taken into account in future metal uptake studies. The fact that the apparent adjustment period to transplant stress was at least 4 weeks may limit the usefulness of this species as a biomonitoring tool since shorter exposure periods on the order of 30 d are desired. Despite the apparent difficulties with interpreting the biological results, the effect of the metal mixture on the distribution of Cd in water and sediment indicates that metal mixtures should be considered in predicting the risk posed by Cd in the aquatic environment.

CHAPTER 7. SUMMARY AND CONCLUSIONS

Molluscs as biomonitoring tools

Based on a review of laboratory and field studies, bivalves (e.g. unionids) were recommended as biomonitors of exposure to metals (Cd, Cu, Zn, Pb, Ni, Hg, As, Cr and Hg) to confirm spatial and temporal changes in bioavailable metals in the environment resulting from mining activities and to determine the effectiveness of remedial measures to improve waste-water treatment. Molluscs should be used in conjunction with several other organisms (e.g. invertebrates, fish, plants) and abiotic measurements (water chemistry, metals in water and sediment) to provide a comprehensive assessment of the overall integrity of the aquatic ecosystem exposed to metals. Further research on the relationship between tissue-residue levels and metal-induced effects are recommended.

Metal mixtures

The metal mixture (Cu, Zn, Pb and Ni) was found to influence the distribution of Cd experimentally added to water and sediment. In the water, the metal mixture was associated with longer residence times for Cd in the water column. Despite the increased potential for Cd accumulation, proportionally less Cd was found in the mussel *Pyganodon grandis* relative to the concentration of the metal mixture. The observed decrease in Cd uptake in the presence of the other metals may have resulted from competition for binding sites on the mussel. In the sediment, Cd shifted from a preferred sorbent (Mn-oxides), but considered more bioavailable, onto other, less preferred sorbents (Fe-oxides and organic matter) and became less bioavailable. The accumulation of Cd by the isoetid macrophyte, *Eriocaùlon septangulare*, could not be explained by the effect of the metal mixture in causing the redistribution of Cd onto the sediment fractions of varying bioavailability.

CHAPTER 8. CONTRIBUTION TO KNOWLEDGE

The most important contribution of this thesis to science is its examination of metal mixtures in natural systems. Metal mixtures have been extensively studied in the laboratory, but few studies have been conducted in the field. The two experiments presented here brought an element of environmental realism to our current understanding of metal mixtures and provided an opportunity for laboratory - field comparisons. Furthermore, these results contribute to our understanding of how different factors affect the bioavailability of metals in the natural environment, which is critical for predicting risk posed by Cd in the aquatic environment.

CHAPTER 9. REFERENCES

Adams, T.G., Atchison, G.J. and Vetter, R.J. 1981. The use of the three-ridge clam (*Amblema perplicata*) to monitor trace metal contamination. Hydrobiologia. **83**: 67-72.

AQUAMIN. 1996. Assessment of the aquatic effects of mining in Canada. Prepared for Aquamin Steering Group. 127 pp.

Atkinson, A.W., Gunning, B.E.S. and John, P.C.L. 1972. Sporopollenin in the cell wall of *Chlorella* and other algae: Ultrastructure, chemistry, and incorporation of ^{14}C-acetate, studied in synchronous cultures. Planta **107**: 1-32.

Balistrieri, L.S. and Murray, J.W. 1986. The surface chemistry of sediments from the Panama Basin: The influence of Mn oxides on metal adsorption. Geochemica Cosmochimica Acta. **50**: 2235-2243.

Bendell Young, L., Dutton, M., and Pick, F.R. 1992. Contrasting two methods for determining trace metal partitioning in oxidized lake sediments. Biogeochemistry **17**: 205-219.

Benjamin, M.M., and Leckie, J.O. 1980. *In* Contaminants and sediments. *Edited by* R. A. Baker. Ann Arbor Science, Ann Arbor, MI. Vol. 2.

Bias, R. and Karbe, L. 1985. Bioaccumulation and partitioning of cadmium within the freshwater mussel *Dreissena polymorpha Pallas*. Int. Rev. Gesamten Hydrobiol. **70**: 113-115.

Borchardt, T. 1985. Relationship between carbon and cadmium uptake in *Mytilus edulis*. Mar. Biol. **85**: 233-244.

Borgmann, U., Norwood, W.P. and Babirad, I.M. 1991. Relationship between chronic toxicity and bioaccumulation of cadmium in *Hyallela azteca*. Can. J. Fish. Aquat. Sci. **48**: 1055-1060.

Boston, H.L. 1986. A discussion of the adaptations for carbon acquisition in relation to the growth strategy of aquatic isoetids. Aquat. Bot. 26: 259-270.

Boston, H.L., Adams, M.S., and Pienkowski, T.P. 1987. Utilization of sediment CO_2 by selected North American isoetids. Ann. Bot. **60**: 485-494.

Boyden, L.D. 1977. Effects of size upon metal content of shellfish. J. Mar. Biol. Ass. **57**: 675-714.

Brix, H. and. Lyngby, J.E. 1985. The influence of size upon the concentrations of Cd, Cr, Cu, Hg, Pb and Zn in the common mussel (*Mytilus edulis* L.). Symp. Biol. Hung. **29**: 253-71.

Brunskill, G.J., and Schindler, D.W. 1971. Geography and bathymetry of selected lake basins, Experimental Lakes Area, Northwestern Ontario. J. Fish. Res. Board Can. **28**: 139-155.

Cain, D.J. and Luoma, S.N. 1990. Influence of Seasonal Growth, Age, and environmental exposure on Cu and Ag in a bivalve indicator, *Macoma balthica*, in San Francisco Bay. Mar. Ecol. Prog. Ser. **60**: 45-55.

Campbell, P.G.C. 1995. Interactions between trace metals and aquatic organisms: A critique of the free-ion activity model. *In* Metal speciation and bioavailability in aquatic systems. *Edited by* A. Tessier and D. R. Turner. John Wiley & Sons Ltd. pp. 45-102.

Campbell, P.G.C. and Stokes, P.M. 1985. Acidification and toxicity of metals to aquatic biota. Can. J. Fish. Aquat. Sci. **42**: 2034-2049.

Campbell, P.G.C. and Tessier, A. 1996. Ecotoxicology of metals in the aquatic environment: geochemical aspects. *In* Ecotoxicology. A hierarchical treatment. *Edited by* M.C. Newman and C.H. Jagoe. Lewis Publishers, Chelsea, MI. pp. 11-58.

Campbell, P.G.C., Tessier, A., Bisson, M., and Bougie, R. 1985. Accumulation of copper and zinc in the yellow water lily, *Nuphar variegatum*: Relationships to metal partitioning in the adjacent lake sediments. Can. J. Fish. Aquat. Sci. **42**: 23-32.

CCREM (Canadian Council of Resource and Environment Ministers). 1987. Canadian water quality guidelines. Task force on water quality guidelines. Environment Canada, Water Quality Objectives Division, Ottawa, ON.

Chapman, P.M., Allen, H.E., Godtredsen, K., and Z'Graggen, M.N. 1996. Evaluation of bioaccumulation factors in regulating metals. Environ. Sci. Tech. **30(10)**: 448-52.

Clark, K. E., Gobas, F.A.P.C., and MacKay, D. 1990. Model of organic chemical uptake and clearance by fish from food and water. Environ. Sci. Tech. **24**: 1203-13.

Clarke, A. 1981. The freshwater molluscs of Canada. Ottawa, Canada: National Museums of Canada. 446 p.

Cossa, D., Bougget, E., Pouliot, D., Piuze, J., and Chanut, J. P. 1980. Geographical and seasonal variations in the relationship between trace metal content and body weight in *Mytilus edulis*. Mar. Biol. (Berlin) **58**: 7-14.

Couillard, Y. 1996. Technical evaluation of metallothionein as a biomarker for the mining industry. CANMET, Natural Resources Canada.191 p.

Couillard, Y., Campbell, P.G.C., and Tessier, A. 1993. Response of metallothionein concentrations in a freshwater bivalve (*Anodonta grandis)* along an environmental cadmium gradient. Limnol. Oceanogr. **38**: 299-313.

Couillard, Y., Campbell, P.G.C., Tessier, A., Pellerin-Massicotte, J., and Auclair, J.C. 1995a. Field transplantation of a freshwater bivalve, *Pyganodon grandis,* across a metal contamination gradient. I. Temporal changes in metallothionein concentrations in soft tissues. Can. J. Fish. Aquat. Sci. **52**: 690-702.

Couillard, Y., Campbell, P.G.C., Pellerin-Massicotee, J., and Auclair, J.C. 1995b. Field transplantation of a freshwater bivalve, *Pyganodon grandis*, across a metal contamination gradient. II. Metallothionein response to Cd and Zn exposure, evidence for cytoxicity, and links to effects at higher levels of biological organization. Can. J. Fish. Aquat. Sci. **52**: 703-715.

Cowan, C. E., Zachara, J. M., and Resch, C. T. 1991. Cadmium adsorption on iron oxides in the presence of alkaline-earth elements. Environ. Sci. Technol. **25**: 437-446.

Crawford, K. and Luoma, S.N. 1993. Guidelines for studies of contaminants in biological tissues for the National Water-Quality Assessment Program. Open-File Report 92-494. U.S. Geological Survey.

Czarnezki, J.M. 1987. Use of the pocketbook mussel, *Lampsilis ventricosa*, for monitoring heavy metal pollution in an Ozark stream. Bull. Environ. Contam. Toxicol. **38**: 641-646.

Davenport, J., and Chen, X. 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). J. Moll. Stud. **53**: 293-297.

Decho, A.W. and Luoma, S.N. 1991. Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica*: signficance to the absorption of carbon and chromium. Mar. Ecol. Prog. Ser. **78**: 303-314.

Decho, A.W. and Luoma, S.N. 1994. Humic and Fulvic Acids: Sink or source in the availability of metals to the marine bivalves *Macoma balthica* and *Potamocorbula amurensis*. Mar. Ecol. Prog. Ser. **108**: 133-45.

Decho, A.W. and Luoma, S.N. 1996. Flexible digestion strategies and trace metal assimilation in marine bivalves. Limnol. Oceanogr. **41(3)**: 568-572.

Department of Fisheries and Oceans. 1986. Policy for the management of fish habitat. Communications Directorate, Department of Fisheries and Oceans, Ottawa, Ontario. 28 pp.

Doherty, F.G., Cherry, D.S., and Cairns, J., Jr. 1987. Valve closure responses of the Asiatic clam *Corbicula fluminea* exposed to cadmium and zinc. Hydrobiologia **153**: 159-167.

Downing, W.L, Shostell, J., and Downing, J.A. 1992. Non-annual external annuli in the freshwater mussels *Anodonta grandis grandis* and *Lamsilis radiata siliquoidea.* Freshwater Biol. **28**: 309-317.

EIFAC. 1987. Water quality criteria for European freshwater fish. EIFAC (European Inland Fish. Adv. Comm.) Tech. Paper. 37. Rev. 1. 75 p.

Elder, J.F. and Collins, J.J. 1991. Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface-water systems. Rev. Environ. Contam. Toxicol. **122**: 37-79.

Elliot, N.G., Swain, R., and Ritz, D.A. 1986. Metal interactions during accumulation by the mussel *Mytilus edulis planulatus*. Mar. Biol. **93**: 395-399.

Enserink, E.L., Maas-Diepeveen, J.L., and van Leeuwen, C.J. 1991. Combined effects of metals: An ecotoxicological evaluation. Wat. Res. **25**: 679-687.

Fisher, N. S., Teyssie, J-L, Fowler, S. W., and Wang, W-X. 1996. Accumulation and retention of metals in mussels from food and water: A comparison under field and laboratory conditions. Environ. Sci. Tech. **30**: 3232-3242.

Flessas, C., Couillard, Y., Pinel-Alloul, B., St-Cyr, L. and Campbell, P.G.C. Relationships between trace metal concentrations in two freshwater gastropods (Mollusca) and environmental contamination in two fluvial lakes of the St. Lawrence River.

France, R.L. 1987. Aggregation in littoral amphipod populations: transformation controversies revisited. Can. J. Fish. Aquat. Sci. **42**: 1510-1515.

France, R.L. and Stokes, P.M. 1988. Isoetid-zoobenthos associations in acid-sensitive lakes in Ontario, Canada. Aquat. Bot. **32**: 99-114.

George, S.G. 1990. Biochemical and cytological assessments of metal toxicity in marine animals. *In* Heavy metals in the environment. *Edited by* R.W. Furness and P.S. Rainbow. CRC Press Inc., Boca Raton, Florida. pp.123-142.

Golberg, E.D., Bowen, V.T., Farrington, J.W., Harvey, G., Martin, J.H., Parker, P.L., Risebrough, R.W., Robertson, W., Schneider, E., and Gamble, E.. 1978. The Mussel Watch. Environ. Conserv. **5(2)**:101-25.

Graney, R.L., Cherry, D.S., and Cairns, J. Jr. 1984. The influence of substrate, pH, diet, and temperature upon cadmium accumulaton in the Asiatic clam (*Corbicula fluminea*) in laboratory artificial streams. Wat. Res. **18(7)**: 833-42.

Green, R.H., Bailey, R.C., Hinch, S.G., Metcalfe, J.L., and Young, V.H. 1989. Use of freshwater mussels (Bivalvia: Unionidae) to monitor the nearshore environment of lakes. J. Great Lakes Res. **15(4)**: 635-44.

Green, R.H., Shiva, S.M., and Bailey, R.C. 1985. Bivalve molluscs as response systems for modeling spatial and temporal environmental patterns. Sci. Tot. Environ. **46**: 147-69.

Hanson, J.M., Mackay, W.C., and Prepas, E.E. 1988. Population size, growth, and production of a unionid clam, *Anodonta grandis simpsoniana*, in a small, deep boreal forest lake in central Alberta. Can. J. Zool. **66**: 247-53.

Hare, L., Carignan and Huerta-Diaz, M.A. 1994. A field experimental study of metal toxicity and accumulation by benthic invertebrates: implications for the acid volatile sulfide (AVS) model. Limnol. Oceanogr. **39**: 1653-1668.

Harris, J.E., Fabris, G.J., Statham, P.J., and Tawfik, F. 1979. Biogeochemistry of selected heavy metals in western port, Victoria, and use of invertebrates as indicators with emphasis on *Mytilus edulis planulatus*. Aust. J. Mar. Freshwater Res. **30**: 159-78.

Harrison, S.E. and Klaverkamp, J.F. 1990. Metal contamination in liver and muscle of northern pike (*Esox lucius*) and white sucker (*Catostomus commersoni*) and in sediments at Flin Flon, Manitoba. Environ. Toxicol. Chem. **9**: 941-956.

Hart, B.T. 1982. Uptake of trace metals by sediments and suspended particulates: a review. Hydrobiol. **91**:299-313.

Harvey, R.W. and Luoma, S.N.. 1985a. Separation of solute and particulate vectors of heavy metal uptake in controlled suspension-feeding experiments with *Macoma balthica*. Hydrobiologia **151**: 97-102.

Harvey, R.W. and Luoma, S.N. 1985b. Effect of adherent bacteria and bacterial extracellular polymers upon assimilation by *Macoma balthica* of sediment-bound Cd, Zn and Ag. Mar. Ecol. Prog. Ser. **22**: 281-289.

Hemelraad, J., Holwerda, D.A., and Zandee, D.I. 1986. Cadmium kinetics in freshwater clams. I. The pattern of cadmium accumulation in *Anodonta Cygnea*. Arch. Environ. Contam. Toxicol. **15**: 1-7.

Hemelraad, J., Kleinveld, H.A., De Roos, A.M., Holwerda, D.A., and Zandee, D.I. 1987. Cadmium kinetics in freshwater clams. III. Effects of zinc on uptake and distribution of cadmium in *Anodonta cygnea*. Arch. Environ. Contam. Toxicol. **16**: 95-101.

Hesslein, R.H. 1976. An *in situ* sampler for close interval pore water studies. Limnol. Oceanogr. **21**: 912-914.

Hinch, S.G. and Green, R.H. 1989. The effects of source and destination on growth and metal uptake in freshwater clams reciprocally transplanted among south central Ontario lakes. Can. J. Zool. **67**: 855-863.

Hinch, S.G. and Stephenson, L.A. 1987. Size- and age-specific patterns of trace metal concentrations in freshwater clams from an acid-sensitive and a circumneutral lake. Can. J. Zool. **65**: 2439-42.

Hinch, S.G., Bailey, R.C., and Green, R.H. 1986. Growth of *Lampsilis radiata* (Bivalvia: Unionidae) in sand and mud: A reciprocal transplant experiment. Can. J. Fish. Aquat. Sci. **43**: 548-52.

Hitchin, G.G., Wile, I., Miller, G.E., and Yan, N.D. 1984. Macrophyte data from 46 southern Ontario soft-water lakes of varying pH. Ont. Minist. Environ. Data Report DR 84/1.

Howell, E.T. 1990. Effects of the isoetid macrophytes *Eriocaùlon septangulàre* and *Lobelia Dortmanna* on the periphyton of littoral sediments. Can. J. Bot. **68**: 1903-1910.

Hudson, R.J.M, and Morel, F.M.M. 1989. Distinguishing between extra- and intracellular iron in marine phytoplankton. Limnol. Oceanogr. 34: 1113-1120.

Huebert, D.B. 1992. The effect of biological and chemical factors on cadmium uptake and toxicity in Lemna trisulca L. PhD thesis. University of Manitoba, Canada, 176 pp.

Huebert, D.B. and Shay, J. 1992. Zinc toxicity and its interaction with cadmium in the submerged aquatic macrophyte *Lemna trisucla* L. Environ. Toxicol. Chem. **11**: 715-720.

Hurlbert, S.H. 1984. Psuedoreplication and the design of ecological field experiments. Ecological Monographs. **54**: 187-211.

Hutchinson, G.E. 1975. A treatise on limnology III. Limnological botany. Wiley-Interscience, N.Y., 660 pp.

ICCS. 1997. International conference on contaminated sediments: Restoration and management. De Doelen, Rotterdam, Netherlands, 7-11 September 1997. Preprints volumes I and II. International Association of Water Quality.

Jaagumagi, R. 1993. Development of Ontario Provincial sediment quality guidelines for arsenic, cadmium, chromium, copper, lead, manganese, mercury, nickel and zinc. Ontario Ministry of the Environment. 10 pp.

Jackson, L.J. and Kalff, J. 1993. Patterns of metal content of submerged aquatic macrophytes: the role of plant growth form. Freshwater Biol. **29**: 351-359.

Jackson, L.J., Kalff, J., and Rasmussen, J.B. 1993. Sediment pH and redox potential affect the bioavailability of Al, Cu, Fe, Mn, and Zn to rooted aquatic macrophytes. Can. J. Fish. Aquat. Sci. **50**: 143-148.

Jeffries, D.S. and Snyder, W.R.. 1981. Atmospheric deposition of heavy metals in central Ontario. Water Air and Soil Poll. **15**: 127-152.

Jenne, E.A. and Luoma, S.N. 1977. Forms of trace elements in soils, sediments, and associated waters: An overview of their determination and biological availability. *In* Biological implications of metals in the environment. *Edited by* H. Drucker and I.R. Wildung. National Technical Information Center, Springfield, VA, USA, pp. 110-143.

Jenner, H. A., Hemelraad, J., Marquenie, J. M., and Noppert, F. 1991. Cadmium kinetics in freshwater clams (Unionidae) under field and laboratory conditions. Sci. Tot. Environ. **108**:205-14.

Johannson, C., Cain, D.J. and Luoma, S.N. 1986. Variability in the fractionation of Cu, Ag, and Zn among cytosolic proteins in the bivalve *Macoma balthica*. Mar. Ecol. Prog. Ser. **28**: 87-97.

Jones, W.G. and Walker, K.F. 1979. Accumulation of iron, manganese, zinc and cadmium by the Australian freshwater mussel *Velesunio ambiguus* (Phillipi) and its potential as a biological monitor. Aust. J. Mar. Freshwater Res. **30**: 741-51.

Jorgensen, C.B., T. Kiorboe, F. Mohlenberg, and H.U. Riisgard. 1984. Ciliary and mucus-net filter feeding with special reference to fluid mechanical characteristics. Mar. Ecol. Prog. Ser. **15**: 283-292.

Kat, P.W. 1984. Parasitism and the Unionacea (Bivalvia). Biol. Rev. **59**: 189-207.

Keller, A.E. and Zam, S.G. 1991. The acute toxicity of selected metals to the freshwater mussel, *Anodonta imbecilis*. Environ. Toxicol. Chem. **10**:539-546.

Kelly, M. 1988. Mining and the freshwater environment. Elsevier Science Publishers Ltd., London. 231 pp.

Kinniburgh, D.G., Jackson, M.L., and Syers, J.K. 1976. Adsorption of alkaline earth, transition, and heavy metal cations by hydrous oxide gels of iron and aluminum. Soil Sci. Soc. Am. Proc. **40**: 796.

Klaverkamp, J.F., Dutton, M.D., Majewski, H.S., Hunt, R.V. and Wesson, L.J. 1991. Evaluation the effectiveness of metal pollution controls in a smelter by using metallothionein and other biochemical responses in fish.. *In* Metal Ecotoxicology Concepts and Applications *Edited by* M.C. Newman and A.W. McIntosh. Lewis Publishers, Chelsea, MI. pp. 33-64.

Kraak, M.H.S., Lavy, D., Schoon, H., Toussaint, M., Peeters, W.H.M., and Van Straalen, N.M. 1994. Ecotoxicity of mixtures of metals to the zebra mussel *Dreissena polymorpha*. Environ. Toxicol. Chem. **13**: 109-114.

Kraak, M.H.S., Scholten, M.C., Peeters, W.H.M., and de Kock, W.C. 1991. Biomonitoring of heavy metals in the Western European Rivers Rhine and Meuse using the freshwater mussel *Dreissena polymorpha*. Environ. Poll. **74**: 101-14.

Kramer, K.J.M., Jenner, H.A. and de Zwart, D. 1989. The valve movement response of mussels: a tool in biological monitoring. Hydrobiologia **188/189**: 433-443.

Langston, W.J. 1980. Arsenic in UK estuarine sediments and its availability to benthic organisms. J. Mar. Biol. Assoc. UK **60**: 869-881.

Langston, W.J. 1982. The distribution of Hg in British estuarine sediments and its availability to deposit-feeding bivalves. J. Mar. Biol. Assoc. UK **62**: 667-684.

Lawrence, S.G. and Holoka, M.H. 1991. Response of crustacean zooplankton *in situ* to cadmium as low environmental concentrations. Verh. Internat. Verein. Limnol. **24**: 2254-2259.

Laxen, D.P.H. 1983. Cadmium adsorption in freshwaters: a quantitative appraisal of the literature. Sci. Total Environ. **30**: 129-146.

Laxen, D.P.H. 1984. Adsorption of Cd, Pb and Cu during the precipitation of hydrous ferric oxide in a natural water. Chem. Geol. **47**: 321-332.

Laxen, D.P.H. 1985. Trace metal adsorption/coprecipitation on hydrous ferric oxide under realistic conditions. Wat. Res. **19**: 1229-1236.

Lemly, A.D. 1993. Teratogenic effects of selenium in natural populations of freshwater fish. Ecotoxico. Environ. Safety 26: 181-204.

Livett, E.A. 1988. Geochemical monitoring of atmospheric heavy metal pollution: theory and applications. Adv. Ecol. Res. **18**: 65: 177.

Lobel, P.B., Bajdik, C.D., Belkhode, S.P., Jackson, S.E. and Longerich, H.P. 1991. Improved protocol for collecting mussel watch specimens taking into account sex, size, condition, shell shape and chronological age. Arch. Environ. Contam. Toxicol. **21**: 409-414.

Lobel, P.B., Belkhode, S.P., Jackson, S.E. and Longerich, H.P. 1989. A universal method for quantifying and comparing the residual variability of element concentrations in biological tissues using 25 elements in the mussel *Mytilus edulis* as a model. Mar. Biol. **102**: 513-518.

Lobel, P.B., Belkhode, S.P., Jackson, S.E., and Longerich, H.P. 1990. Recent taxonomic discoveries concerning the mussel *Mytilus edulis*: Implications for biomonitoring. Arch. Environ. Contam. Toxicol. **19**: 508-512.

Luoma, S. N. 1983. Bioavailability of trace metals to aquatic organisms - A Review. Sci. Tot. Environ. **28**: 1-22.

Luoma, S. N. 1996. The developing framework of marine ecotoxicology: Pollutants as a variable in marine ecosystems. J. Exp. Mar. Biol. and Ecol. 200: 29-55.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms - A review. Sci. Total Environ. **28**:1-22.

Luoma, S.N. and Bryan, G.W. 1978. Factors controlling the availability of sediment-bound lead to the estuarine bivalve *Scrobicularia plana*. J. Mar. Biol. Assoc. UK **58**: 793-802.

Luoma, S.N. and Bryan, G.W. 1981. Statistical assessment of the form of trace metals in oxidized estuarine sediments employing chemical extractants. Sci. Total Environ. **17**: 165-196.

Luoma, S.N. and Carter, J.L. 1991. Effects of trace metals on aquatic benthos. *In* Metal Ecotoxicology. Concepts and applications. *Edited by* M.C. Newman and A.W. McIntosh. Lewis Publishers, Chelsea, Mich. pp. 261-300.

Luoma, S.N. and Fisher, N. 1997. Uncertainties in assessing contaminant exposure from sediments. In Critical Issues in Methodological Uncertainty. Edited by C.G. Ingersoll, T. Dillon and G.R. Biddinger. SETAC Special Publication Series. Lewis Publishers, Boca Raton, FL. pp. 211-237.

Luoma, S.N. and Jenne, E.A. 1976. Factors affecting the availability of sediment-bound cadmium to the estuarine, deposit-feeding clam, *Macoma balthica*. *In* Radioecology and energy resources. *Edited by* C.E. Cushing. Ecol. Soc. Am. Spec. Publ. No. 1, Dowden, Hutchinson and Ross, Stroudsbourg, PA, pp. 283-290.

Luoma, S.N. and Jenne, E.A. 1977. The availability of sediment-bound cobalt, silver and zinc to a deposit-feeding clam. *In* Biological implications of metals in the environment. *Edited by* H. Drucker and R.E. Wildung. ERDA Symp. Ser. No. 42, Energy Research and Development Administration, Washington, DC. pp. 213-230.

Luoma, S.N., Johns, C., Fisher, N.S., Steinberg, N.A., Oremland, R.S., and Reinfelder, J.R. 1992. Determination of selenium bioavailability to a benthic bivalve from particulate and dissolved pathways. Environ. Sci. Technol. **26**: 485-491.

Malley, D.F. 1996. Cadmium whole-lake experiment at the Experimental Lakes Area: an anachronism? Can. J. Fish. Aquat. Sci. **53**: 1862-1870.

Malley, D.F. and Mills, K.H. 1992. Whole-lake experimentation as a tool to assess ecosystem health, response to stress and recovery: the Experimental Lakes Area experience. J. Aquat. Ecosyst. Health **1**: 159-174.

Malley, D.F., Chang, P.S.S., and Hesslein, R.H. 1989. Whole lake addition of cadmium-109: Radiotracer accumulation in the mussel population in the first season. Sci. Total Environ. **87/88**: 397-417.

Malley, D.F., Hubner, J.D. and Donkersloot, K. 1988. Effects of ionic composition of blood and tissues of *Anodonta grandis grandis* (Bivalvia) of an addition of aluminum and acid to a lake. Arch. Environ. Contam. Toxicol. **17**: 479-491.

Malley, D.F., Klaverkamp, J.F., Brown, S.B., and Chang, P.S.S. 1993. Increase in metallothionein in freshwater mussels *Anodonta grandis grandis* exposed to cadmium in the laboratory and the field. Wat. Pollut. Res. J. Can. **28**: 253-273.

Malley, D.F., Stewart, A.R., and Hall, B.D. 1995. Uptake of methyl mercury by the floater mussel, *Anodonta grandis grandis* (Bivalvia, Unionidae) caged in a flooded wetland. Environ. Toxicol. Chem. **15**: 928-936.

Manly, R. and George, W.O. 1977. The occurrence of some heavy metals in populations of the freshwater mussel *Anodonta anatina* (L.) from the river Thames. Environ. Pollut. **14**: 139-154.

McCorkle, S., Shirley, T.C., and Dietz, T.H. 1979. Rhythms of activity and oxygen consumption in the common pond clam *Ligumia subrostrata* (Say). Can. J. Zool. **57**: 1960-1964.

McMahon, R.F. 1991. Mollusca: Bivalvia. *In* Ecology and Classification of North American Freshwater invertebrates. *Edited by* J. H. Thorp and A. P. Covich. Academic Press, Inc., San Diego, California. pp. 315-99.

Mersch, J., Wagner, P., and Pihan, J-C. 1996. Copper in indigenous and transplanted zebra mussels in relation to changing water concentrations and body weight. Environ. Toxicol. Chem. **15(6)**: 886-93.

Metcalfe-Smith, J.L. 1994. Influence of species and sex on metal residues in freshwater mussels (Family Unionidae) from the St. Lawrence River, with implications for biomonitoring. Environ. Toxicol. Chem. **13(9)**:1433-43.

Metcalfe-Smith, J.L. and Green, R.H. 1992. Ageing studies on three species of freshwater mussels from a metal-polluted watershed in Nova Scotia, Canada. Can. J. Zool. **70**: 1284-91.

Metcalfe-Smith, J.L., Green, R.H., and Grapentine, L.C. 1996. Influence of biological factors on concentrations of metals in the tissues of freshewater mussels (*Elliptio complanata* and *Lampsilis radiata radiata*) from the St. Lawrence River. Can. J. Fish. Aquat. Sci. **53**: 205-19.

Metcalfe-Smith, J.L., Merriman, J.C., and Batchelor, S. P. 1992. Relationships between concentrations of metals in sediment and two species of freshwater mussels in the Ottawa River. Water Poll. Res. J. Can. **27(4)**: 845-69.

Moeller, R.E. 1975. Hydrophyte biomass and community structure in a small, oligotrophic New Hampshire lake. Verh. Internat. Verein. Limnol. **19**: 1004-1012.

Monitoring Assessment and Research Centre. 1985. Historical monitoring. MARC Report No. 31. Monitoring Assessment and Research Center, London.

Morel, F.M.M. 1983. Principles of aquatic chemistry. Wiley & Sons Inc., Toronto, ON. 446 p.

Naddy, R.B., La Point, T.W., and Klaine, S.J. 1995. Toxicity of arsenic, molybdenum and selenium combinations to *Ceriodaphnia dubia*. Environ. Toxicol. Chem. **14**: 329-336.

Nieboer, E. and Richardson, D.H.S. 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. Environ. Poll. (Series B) **1**: 3-26.

Nriagu, J.O. 1980. Production, uses, and properties of cadmium. *In* Cadmium in the environment. Part 1. Ecological cycling. *Edited by* J.O. Nriagu. Wiley and Sons, Toronto. pp. 35-70.

Nriagu, J.O. 1990. Global metal pollution. Poisoning the biosphere? Environment **32**: 7-33.

Nriagu, J.O. and Pacyna, J.M. 1988. Quantitative assessment of world-wide contamination of air, water and soils with trace metals. Nature **333**: 134-139.

Nriagu, J.O., Wong, H.K.T., and Coker, R.D. 1982. Deposition and chemistry of pollutant metals in lakes around the smelters at Sudbury, Ontario. Environ. Sci. Technol. **16**: 551-560.

Nyffeler, U.P., Li, Y.H., and Santschi, P.H. 1984. A kinetic approach to describe trace-element distribution between particles and solution in natural aquatic systems. Geochim. Cosmochim. Acta **45**: 2377-2383.

O'Connor. 1992. Recent trends in coastal environmental quality: Results from the first five years of the NOAA Mussel Watch Project. Washington, DC. U.S. Department of Commerce, NOAA, National Ocean Service. 46 p.

Papelis, C., Hayes, K.F., and Leckie, J.O. 1988. HYDRAQL: A program for the computation of chemical equilibrium composition of aqueous batch systems including surface complexation modeling of ion adsorption at the oxide/solution interface. Stanford Univ. Dep. Civ. Eng. Tech. Rep. 306.

Paterson, C.G. 1984. A technique for determining apparent selective filtration in the freshwater unionid bivalve, *Elliptio complanata* (Lightfoot). Veliger **27**: 238-241.

Phillips, D.J.H. and Rainbow, P.S. 1993. Biomonitoring of trace aquatic contaminants. Essex, England: Elsevier Science Publishers Ltd.

Popham, J.D. and D'Auria, J.M. 1983. Combined effect of body size, season, and location on trace element levels in mussels (*Mytilus edulis*). Arch. Environ. Contam. Toxicol. **12**: 1-14.

Pynnönen, K. 1995. Effect of pH, hardness and maternal pre-exposure on the toxicity of Cd, Cu and Zn to the glochidial larvae of a freshwater clam *Anodonta cygnea*. Wat. Res. **29**: 247-254.

Rainbow, P.S. and Dallinger, R. 1993. Metal uptake, regulation and excretion in freshwater invertebrates. *In* Ecotoxicology of metals in invertebrates. *Edited by* R. Dallinger and P.S. Rainbow. SETAC Special Publication Series. Lewis Publishers, Boca Raton, FL. pp. 119-132.

Regoli, F. 1992. Lysosomal responses as a sensitive stress index in biomonitoring heavy metal pollution. Mar. Ecol. Prog. Ser. **84**: 63-69.

Regoli, F. and Orlando, E. 1994. Accumulation and subcellular distribution of metals (Cu, Fe, Mn, Pb and Zn) in the mediterranean mussel *Mytilus galloprovincialis* during a field transplant experiment. Mar. Poll. Bull. 28(10): 592-600.

Reinfelder, J.R. and Fisher, N.S. 1991. The assimilation of elements ingested by marine copepods. Science. **251**: 794-796.

Ritz, D.A., Swain, R., and Elliot, N.G. 1982. Use of the mussel *Mytilus edulis planulatus* (Lamarck) in monitoring heavy metal levels in seawater. Aust. J. Mar. Freshwater Res. **33**: 491-516.

Roberts, D.A., Singer, R., and Boylen, C.W. 1985. The submersed macrophyte communities of Adirondack lakes (New York, USA) of varying degrees of acidity. Aquat. Bot. **21**: 219-235.

Robinson, B.L. and Fernald, M. 1909. Emendations of the seventh edition of Gray's Manual, I. Rhodora **11**: 389-391.

Robinson, W.E. and Ryan, D.K. 1986. Metal interactions within the kidney, gill and digestive gland of the hard clam, *Mercenaria mercenaria*, following laboratory exposure to cadmium. Arch. Environ. Contam. Toxicol. **15**: 23-30.

Roesijadi, G. and Klerks, P.L. 1989. Kinetic analysis of cadmium binding to metallothionein and other intracellular ligands in oyster gills. J. Exp. Zool. **251**: 1-12.

Romeril, M.G. 1979. The occurrence of copper, iron and zinc in the hard shell clam, *Mercenaria mercenaria*, and sediments of South Hampton water. Estuarine Coastal Mar. Sci. **9**: 423-34.

Rosenberg, D.M. and Resh, V.H. 1993. Freshwater biomonitoring and benthic macroinvertebrates. New York, NY: Chapman & Hall Inc. 488 p.

Rule, J.H. and Alden, R.W. III 1992. Partitioning of Cd in geochemical fractions of anaerobic estuarine sediments. Estuarine Coastal Shelf Sci. **34**: 487-499.

Rule, J.H. and Alden, R.W. III 1996a. Interactions of Cd and Cu in anaerobic estuarine sediments. I. Partitioning in geochemical fractions of sediments. Environ. Toxicol. Chem. **15**: 460-465.

Rule, J.H. and Alden, R.W. III 1996b. Interactions of Cd and Cu in anaerobic estuarine sediments. I. Bioavailability, body burdens and respiration effects as related to geochemical partitioning. Environ. Toxicol. Chem. **15**: 466-471.

Russell-Hunter, W.D. 1983. Ecology. Vol 6. *In* The Mollusca. *Edited by* K.M. Wilbur. Orlando, Florida: Academic Press Inc. 695 p.

Salánki, J, V.-Balogh, K., Berta, E. 1982. Heavy metals in animals of Lake Balaton. Water Res. **16**: 1147-1152.

Salánki, J. and V.-Balogh, K. 1989. Physiological background for using freshwater mussels in monitoring copper and lead pollution. Hydrobiologia. **188/189**: 445-454.

Salazar, S.M., Beckvar, N., Salazar, M.H., and Finkelstein, K. 1996. An in-situ assessment of mercury contamination in the Sudbury River, Massachusetts, using bioaccumulation and growth in transplanted freshwater mussels. NOAA Technical Memorandum NOS ORCA 89. Seattle, Washington: National Oceanic Atmospheric Administration. 66 p.

Salisbury, F.B. and Ross, C.W. 1992. Plant physiology. Fourth edition., Wadsworth, Inc., Belmont, California. 682 pp.

Santschi, P.H., Nyffeler, U.P., Anderson, R.F., Schiff, S.L., and O'Hara, P. 1986. Response of radioactive trace metals to acid-base titrations in controlled experimental ecosystems: evaluation of transport parameters for application to whole-lake radiotracer experiments. Can. J. Fish. Aquat. Sci. **43**: 60-77.

SAS Institute Inc. 1989. SAS/STAT® User's Guide: Version 6, Fourth edition. Volume 2. Cary, NC.

Scheider, W.A., Jeffries, D.S., and Dillon, P.J. 1981. Bulk deposition in the Sudbury and Muskoka-Haliburton areas of Ontario during the shutdown of INCO Ltd in Sudbury. Atm. Environ. **15**: 945-956.

Schindler, D.W. 1988. Experimental studies of chemical stressors on whole lake ecosystems. Verh. Int. Ver. Theor. Angew. Limnol. **23**: 11-41.

Scholz, N. 1980. Accumulation, loss and molecular distribution of cadmium *in Mytilus edulis*. Helgol. Meeresunters. **33**: 68-78.

Seymour, A.H. and Nelson, V.A. 1973. Decline of ^{65}Zn in marine mussels following the shutdown of Hanford reactors. *In* Radioactive contamination of the marine environment, IAEA Report SM-158116. Vienne. International Atomic Energy Agency.

Sigg, L., Sturm, M., and Kistler, D. 1987. Vertical transport of heavy metals by settling particles in Lake Zurich. Limnol. Oceanogr. **32**: 112-130.

Smits, A.J.M, Laan, P., Their, R.H., and van der Velde, G. 1990. Root aerenchyma, oxygen leakage patterns and alcoholic fermentation ability of the roots of some

nymphaeid and isoetid macrophytes in relation to the sediment type of their habitat. Aquat. Bot. **38**: 3-17.

Spehar, R.L. and Fiandt, J.T. 1986. Acute and chronic effects of water quality criteria-based metal mixtures on three aquatic species. Environ. Toxicol. Chem. **5**: 917-931.

Sprung, M. and Rose, U. 1988. Influence of food size and food quality on the feeding of the mussel *Dreissena polymorpha*. Oecologia **77**: 526-32.

Stainton, M.P., Capel, M.J., and Armstrong, F.A.J. 1977. The chemical analysis of freshwater. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25.

St-Cyr, L and Campbell, P.G.C. 1994. Trace metals in submerged plants of the St. Lawrence River. Can. J. Bot. **72**: 429-439.

St-Cyr, L, Campbell, P.G.C., and Guertin, K. 1994. Evaluation of the role of submerged plant beds in the metal budget of a fluvial lake. Hydrobiologia **291**: 141-156.

Stephenson, M and Mackie, G.L. 1988. Total cadmium concentrations in the water and littoral sediments of central Ontario lakes. Water, Air and Soil Poll. **38**: 121-136.

Stephenson, M. and Mackie, G.L. 1989. A laboratory study of the effects of waterborne cadmium, calcium and carbonate concentrations on cadmium concentrations in *Hyalella axteca* (Crustacea: Amphipoda). Aquat. Toxicol. **15**: 53-62.

Stephenson, M., Bendell-Young, L., Bird, G.A., Brunskill, G.J., Curtis, P.J., Fairchild, W.L., Holoka, M.H., Hunt, R.V., Lawrence, S.G., Motycka, M.F., Schwartz, W.J., Turner, M.A., and Wilkinson, P. 1996. Sedimentation of experimentally added cadmium and ^{109}Cd in Lake 382, Experimental Lakes Area, Canada. Can. J. Fish. Aquat. Sci. **53**: 1888-1902.

Stewart, A.R. 1997. Technical evaluation of molluscs as a biomonitoring tool for the Canadian mining industry. Canadian Centre for Mineral and Energy Technology, Natural Resources Canada. 248 pp.

Stewart, A.R. and D.F. Malley. 1994. Accumulation of cadmium is affected by the presence of other metals. Abstract Book of the 15th Annual meeting of the Society of Environmental Toxicology and Chemistry. SETAC, Pensacola, FL. 282 p.

Stewart-Oaten, A., Murdock, W., and Parker, K.R. 1986. Environmental impact assessment: "Pseudoreplication" in time. Ecology. **67(4)**: 929-40.

Szefer, P. and Szefer, K. 1985. Occurrence of ten metals in *Mytilus edulis* L. and *Cardium glaucum* L. From the Gdansk Bay. Mar. Poll. Bull. **16**: 446-50.

Tankersley, R.A. and Dimock, R.V., Jr. 1993. The effect of larval brooding on the filtration rate and particle retention efficiency of *Pyganodon cataracta* (Bivalvia: Unionidae). Can. J. Zool. **71**: 1934-44.

Tessenow, U. and Baynes, Y. 1978. Experimental effects of *Isoetes lacustris* L. on the distribution of Eh, pH, Fe and Mn in lake sediment. Verh. Int. Ver. Limnol. **20**: 2358-2362.

Tessier, A., Campbell, P.G.C., Auclair, J.C., and Bisson, M. 1984. Relationship between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. Can. J. Fish Aquat. Sci. **41**: 1463-72.

Tessier, A., Campbell, P.G.C., Auclair, J.C., Schenck, R.C., Huizenga, D. et Dubreuil, B. 1987. Contribution à l'amélioration des critères scientifiques pour évaluer la signification biologique de métaux traces dans les sédiments. Environment Canada, No. SB-43031745. 139 pp.

Tessier, A., Couillard, Y., Campbell, P.G.C., and Auclair, J.C. 1993. Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis grandis*. Limnol. Oceanogr. **38**:1-17.

Tessier, L., Vaillancourt, G., and Pazdernik, L. 1994. Comparative study of the cadmium and mercury kinetics between the short-live gastropod *Viviparus georgianus* (Lea) and pelecypod *Elliptio complanata* (Lightfoot), under laboratory conditions. Environ. Poll. **85**: 271-82.

The State of Canada's Environment. 1996. Environment Canada. Cat. No. En21-54/1996E-IN.

Thorp, J.H. and Covich, A.P. 1991. Introduction to Freshwater Invertebrates. *In* Ecology and classification of North American freshwater invertebrates. *Edited by* J. H. Thorp and A. P. Covich. Academic Press Inc., San Diego, California. pp. 1-15.

Underwood, A.J. 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. J. Exp. Mar. Biol. Ecol. **161**: 145-78.

V.-Balogh, K. 1988. Comparison of mussels and crustacean plankton to monitor heavy metal pollution. Wat. Air Soil Poll. **37**: 281-92.

Voyer, R.A. and Heltshe, J.F. 1984. Factor interactions and aquatic toxicity testing. Water Res. **18**: 441-447.

Wagemann, R., Capel, M.J., Hesslein, R., and Stephenson, M. 1994. Sediment-water distribution coefficients and speciation of cadmium in a Canadian Shield lake. Can. J. Fish. Aquat. Sci. **51**: 1951-1958.

Wang, W.X. and Fisher, N. S. 1996a. Assimilation of trace elements and carbon by the mussel *Mytilus edulis*: Effects of food composition. Limnol. Oceanogr. **41(2)**: 197-207.

Wang, W.X. and Fisher, N.S. 1996b. Assimilation of trace elements by the mussel *Mytilus edulis*: effects of diatom chemical composition. Mar. Biol. **125**: 715-724.

Wang, W.X., Fisher, N.S., and Luoma, S.N. 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. **140**: 91-113.

Wang, W.X., Griscom, S.B. and Fisher, N.S. 1997. Bioavailability of Cr(III) and Cr(VI) to marine mussels from solute and particulate pathways. Environ. Sci. Technol. **31**: 603-611.

Widdows, J.D., P. Donkin, M.D. Brinsley, S.V. Evans, P.N. Salkeld, A. Franklin, R.J. Law, M.J. Waldock. 1995. Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. Mar. Ecol. Prog. Ser. **127**: 131-148.

Williams, J.D., Warren, M. L. Jr., Cummings, K.S., Harris, J.L., and Neves, R.J. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries **18(9)**: 6-22.

Wilson, S.D. and Keddy, P.A. 1985. Plant zonation on a shoreline gradient: physiological response curves of component species. J. Ecol. **73**: 851-860.

Wium-Andersen, S. 1971. Photosynthetic uptake of free CO_2 by the roots of *Lobelia dortmanna*. Physiol. Plant. **25**: 245-248.

Wium-Andersen, S. and Andersen, J.M. 1972. Carbon dioxide content in the interstial water in the sediment of Grane Langsø, a Danish Lobelia lake. Limnol. Oceanogr. 17: 943-947.

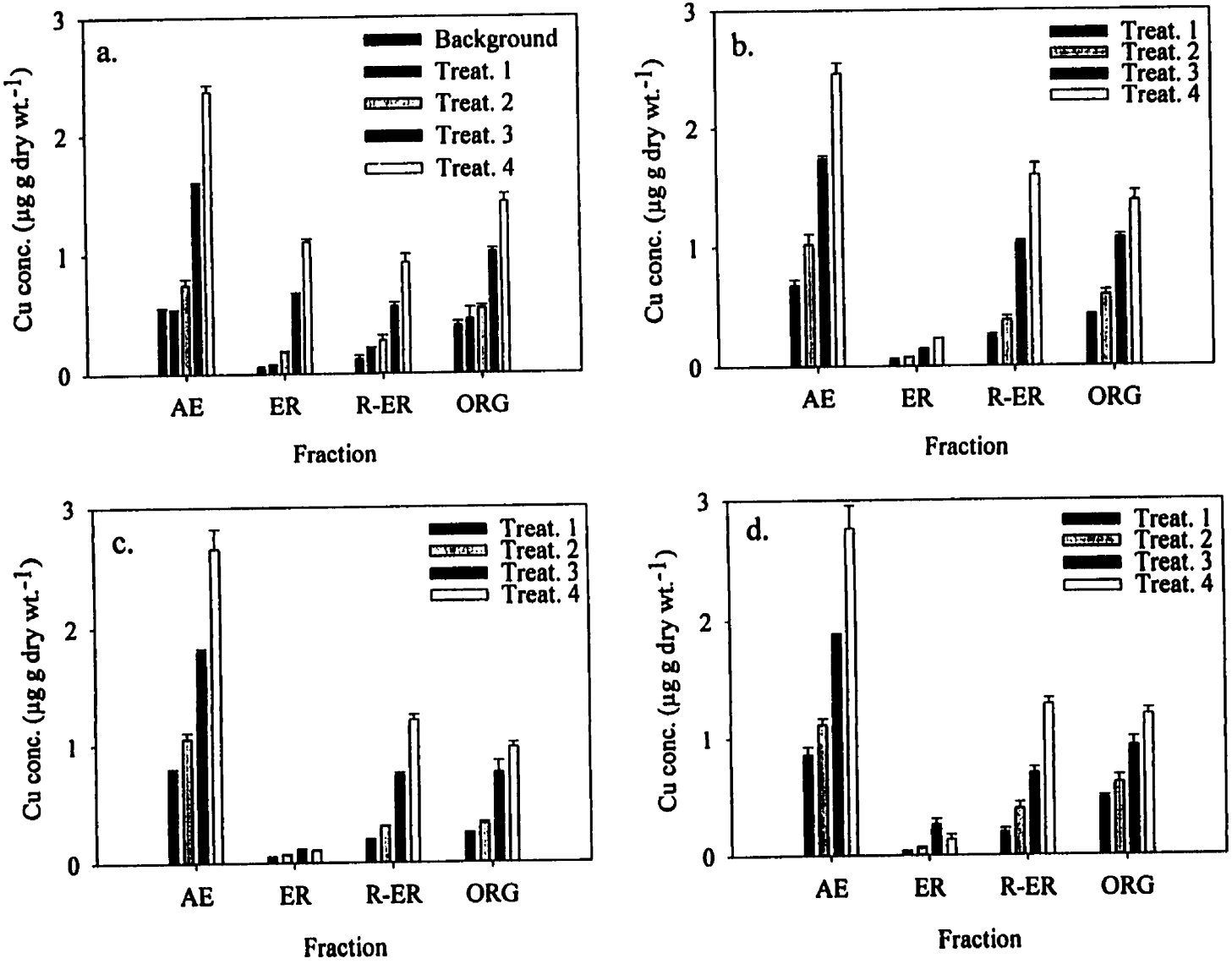
Wolfe, D.A. 1970. Levels of stable Zn and ^{65}Zn in *Crassostrea virginica* from North Carolina. J. Fish. Res. Bd. Can. 27: 47-57.

Wood, T.M., Baptista, A.M., Kuwabara, J.S., and Flegal, A.R. 1995. Diagnostic modeling of trace metal partitioning in south San Francisco Bay. Limnol. Oceanogr. 40: 345-358.

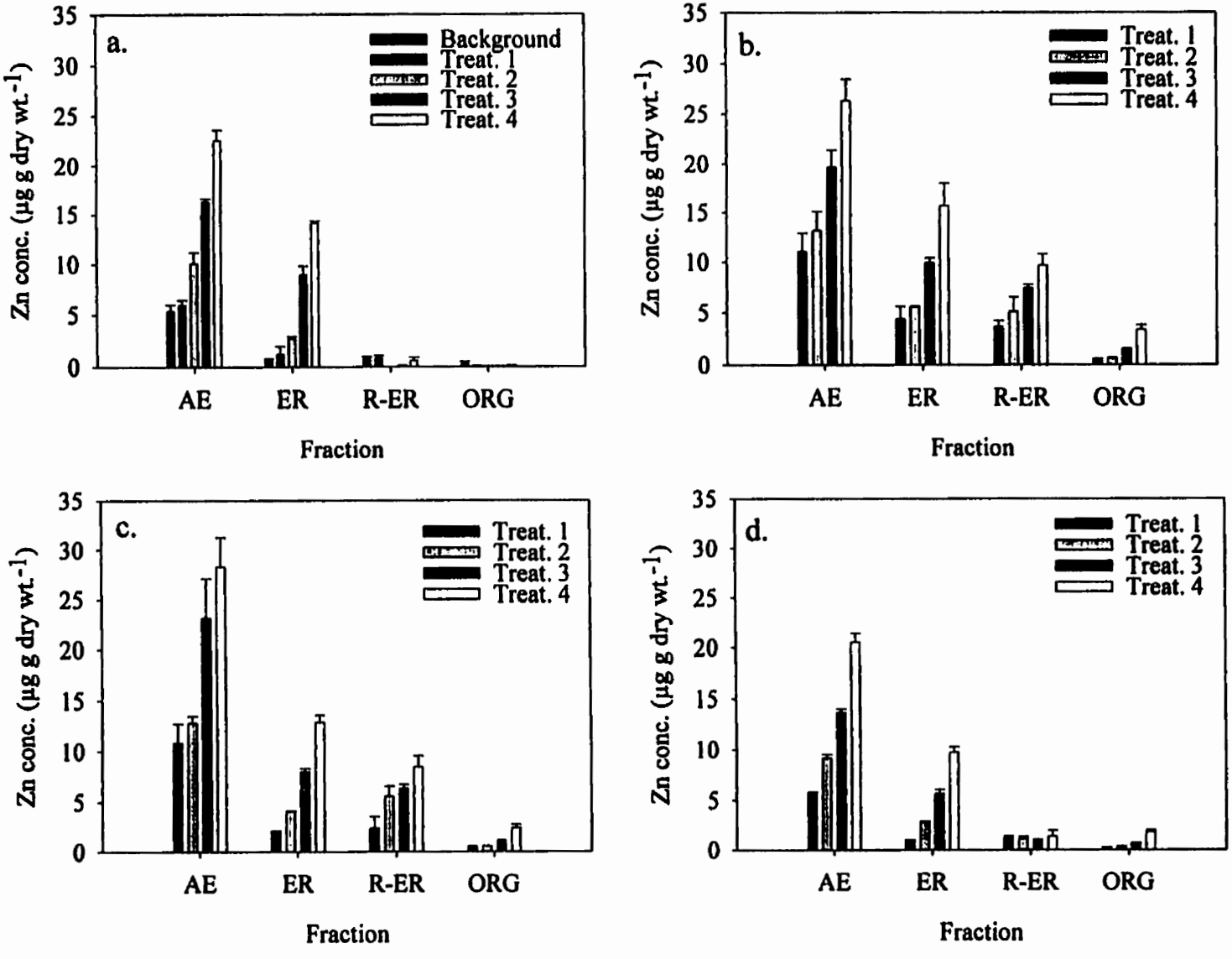
Yan, N.D., Miller, G.E., Wile, I., and Hitchin, G.G. 1985. Richness of aquatic macrophyte floras of soft water lakes of differing pH and trace metal content in Ontario, Canada. Aquat. Bot. 23: 27-40.

Young, D.R. and Folsom, T.R. 1967. Loss of Zn-65 from the California sea-mussel *Mytilus californianus*. Biol. Bull. 133: 438-447.

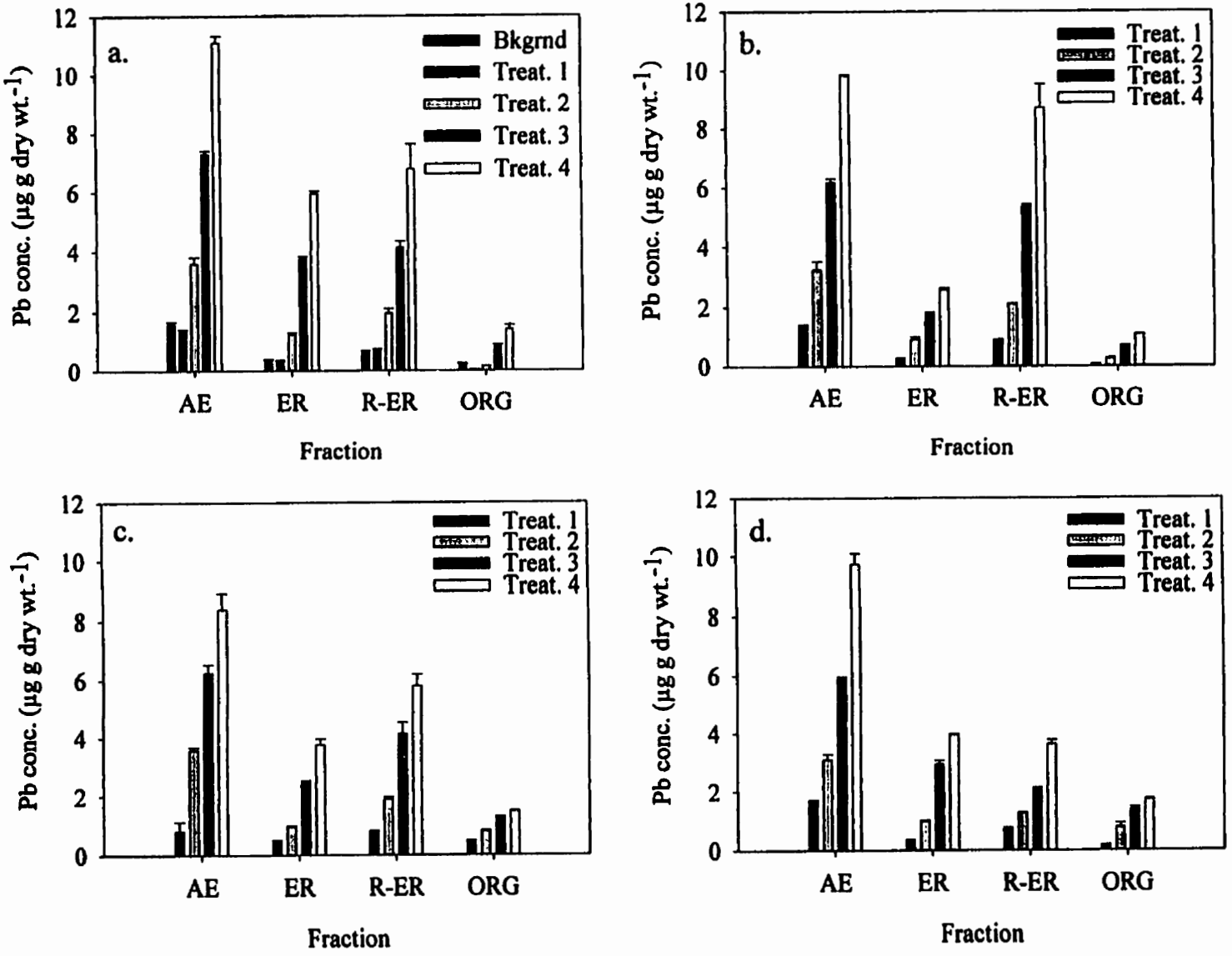
CHAPTER 10. APPENDICES



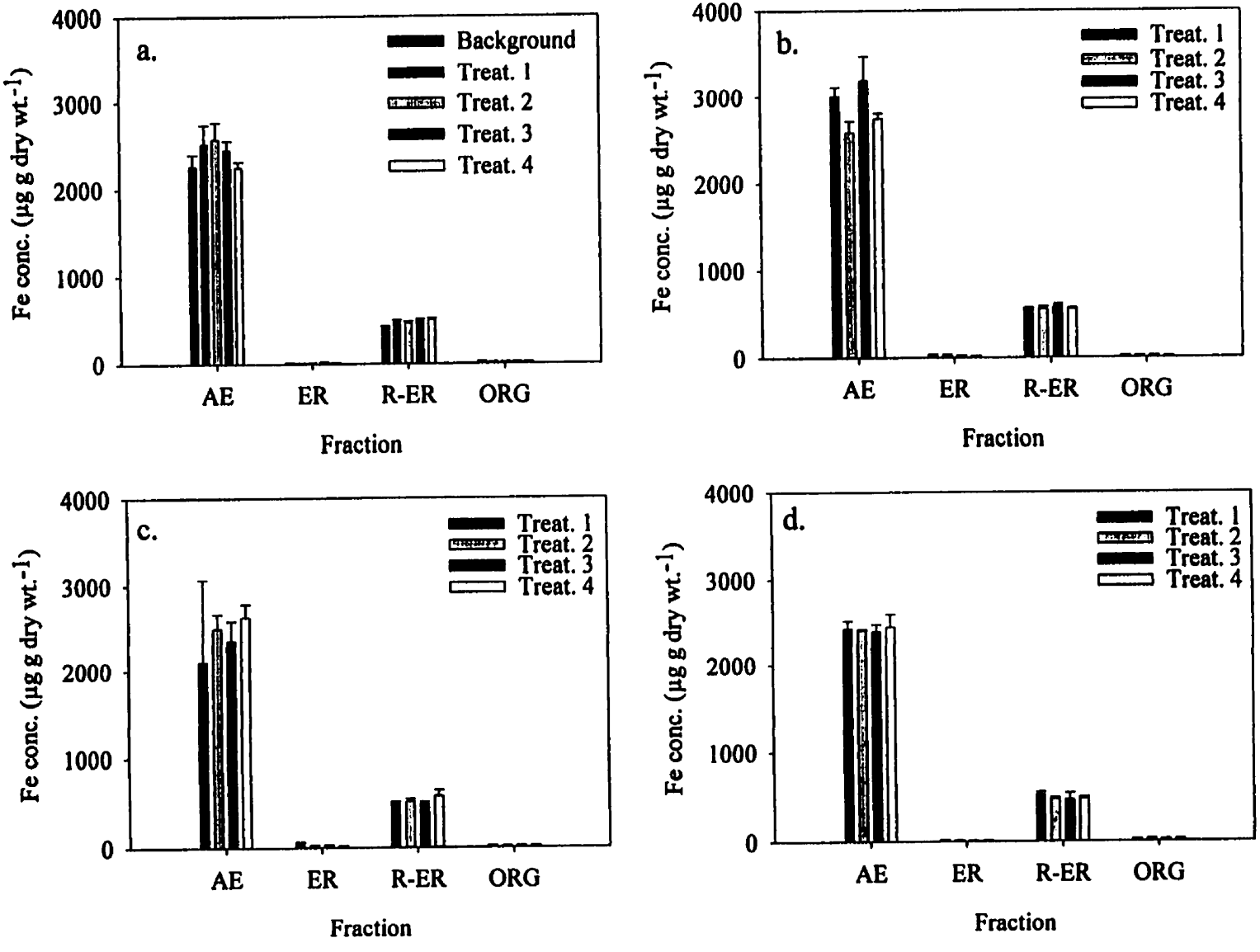
Appendix 10.1 Copper concentration (µg g^{-1}) in each of the geochemical fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



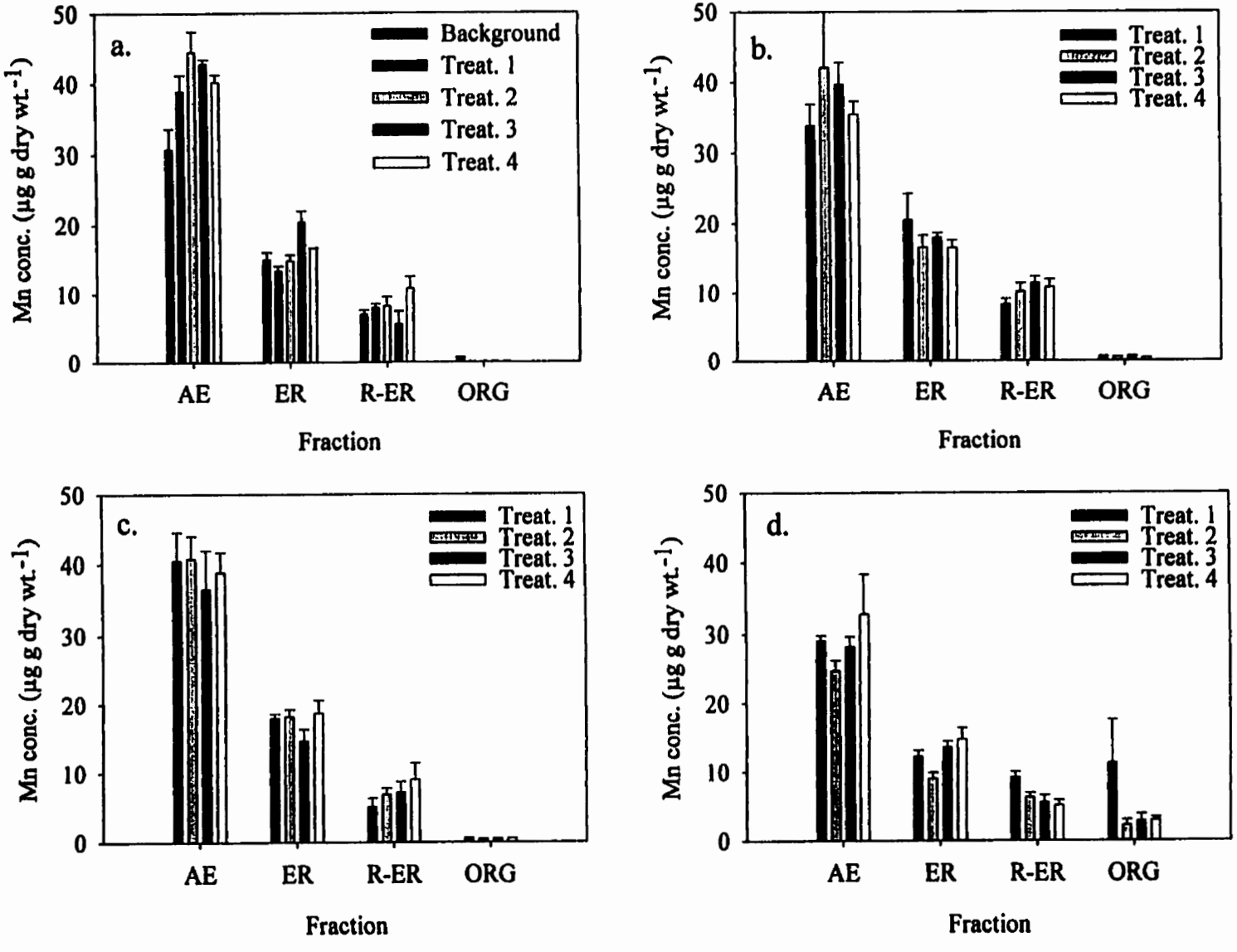
Appendix 10.2 Zinc concentration (µg g^{-1}) in each of the geochemical fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



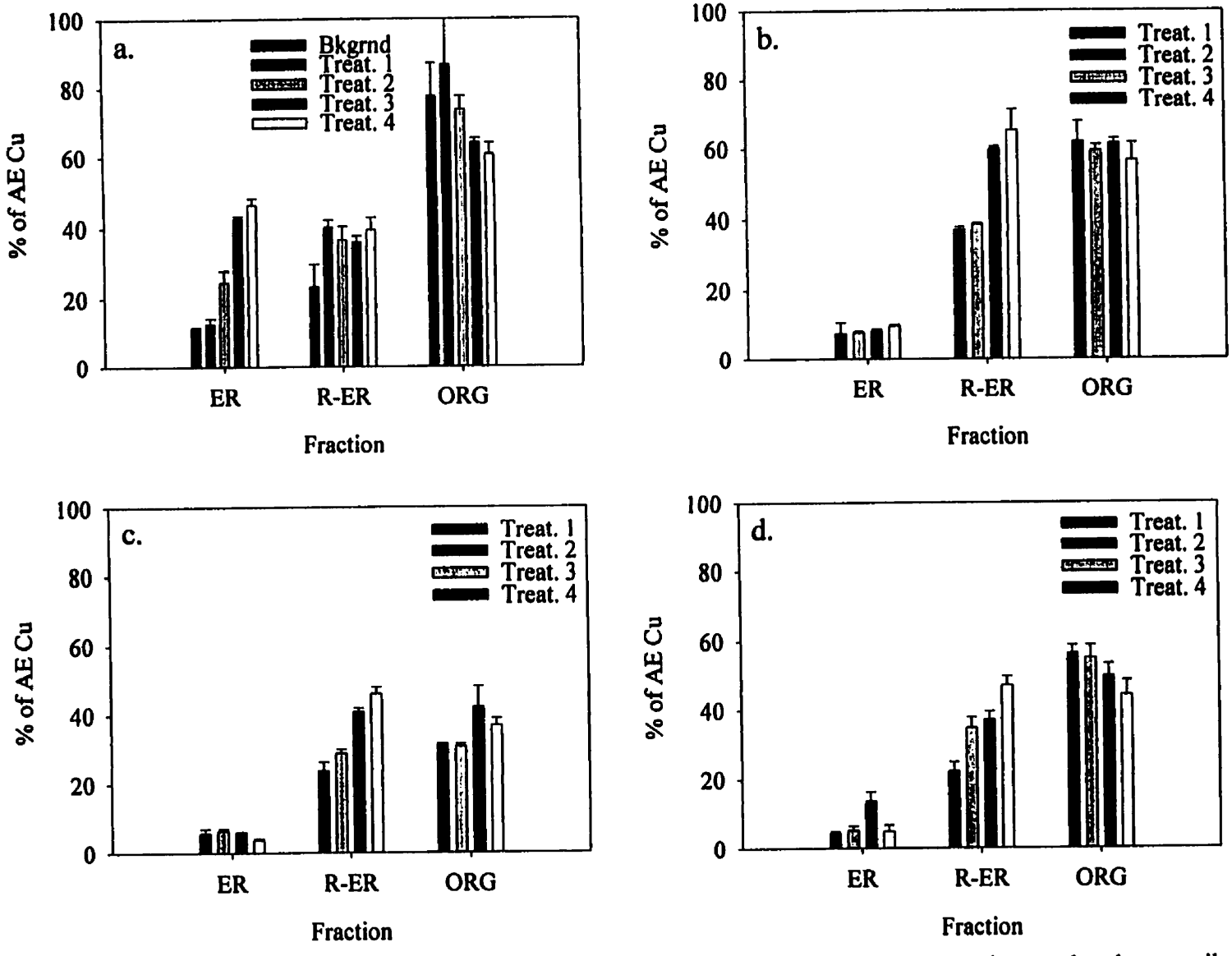
Appendix 10.3 Lead concentration (µg g^{-1}) in each of the geochemical fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



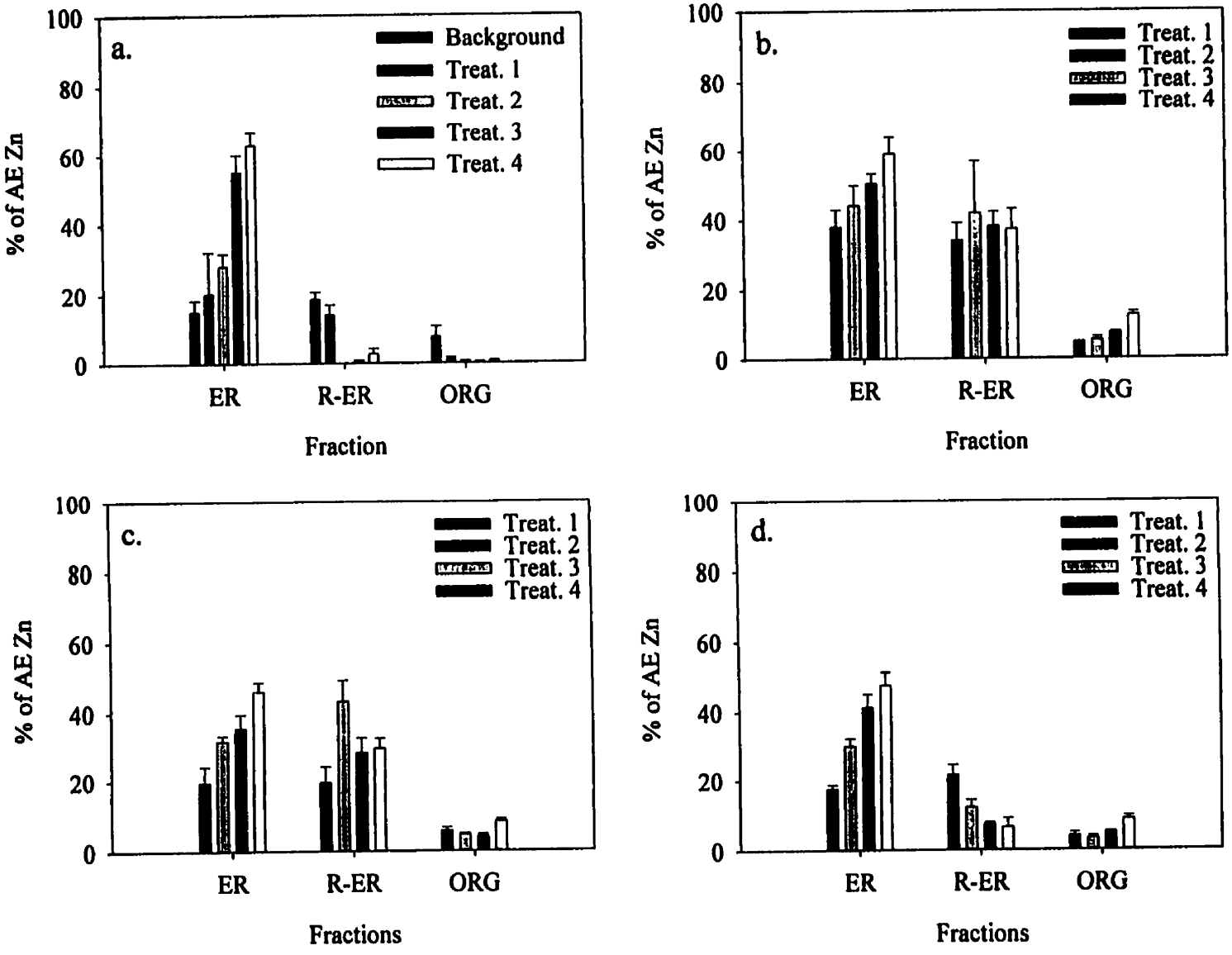
Appendix 10.4 Iron concentration (µg g^{-1}) in each of the geochemical fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



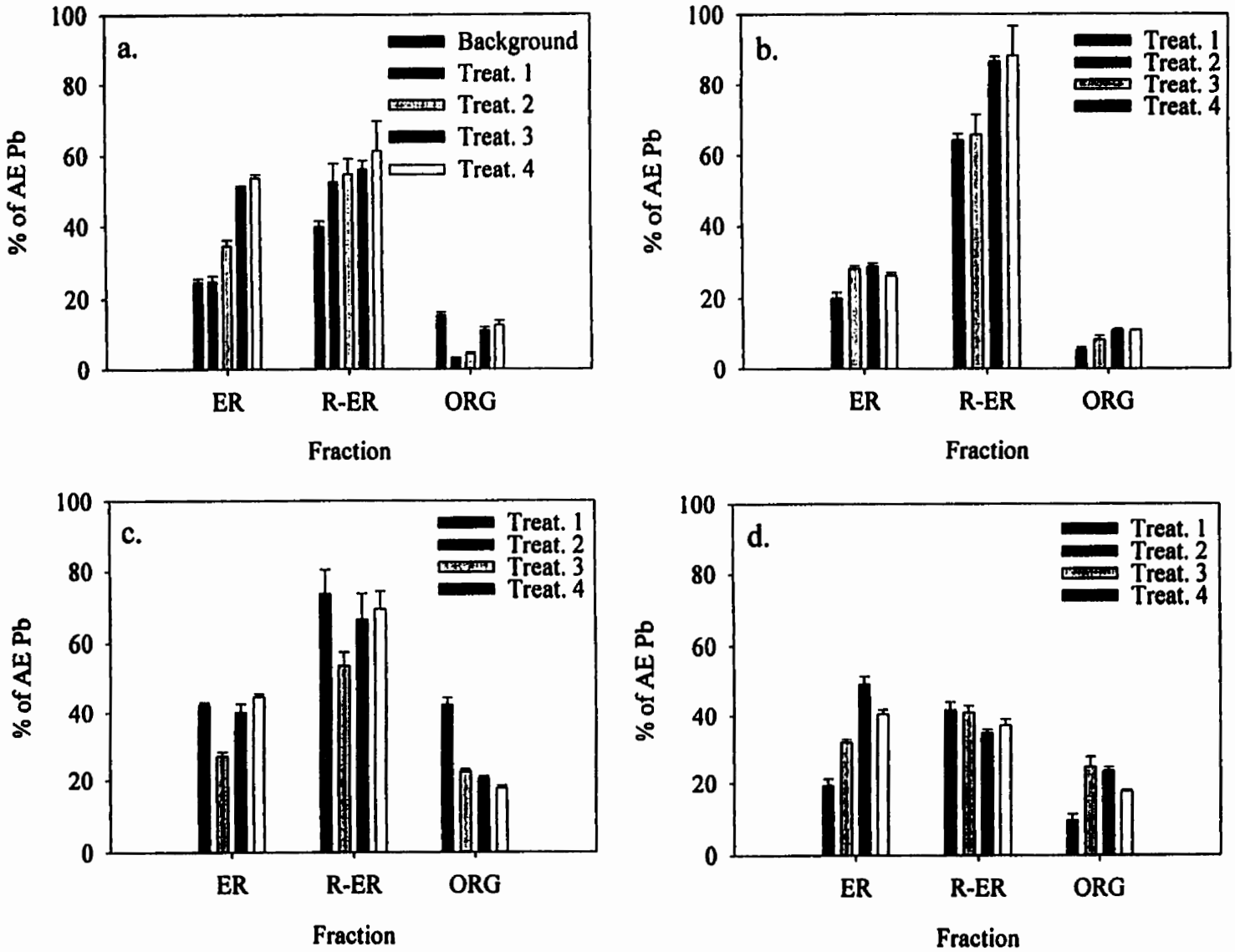
Appendix 10.5 Manganese concentration (µg g^{-1}) in each of the geochemical fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



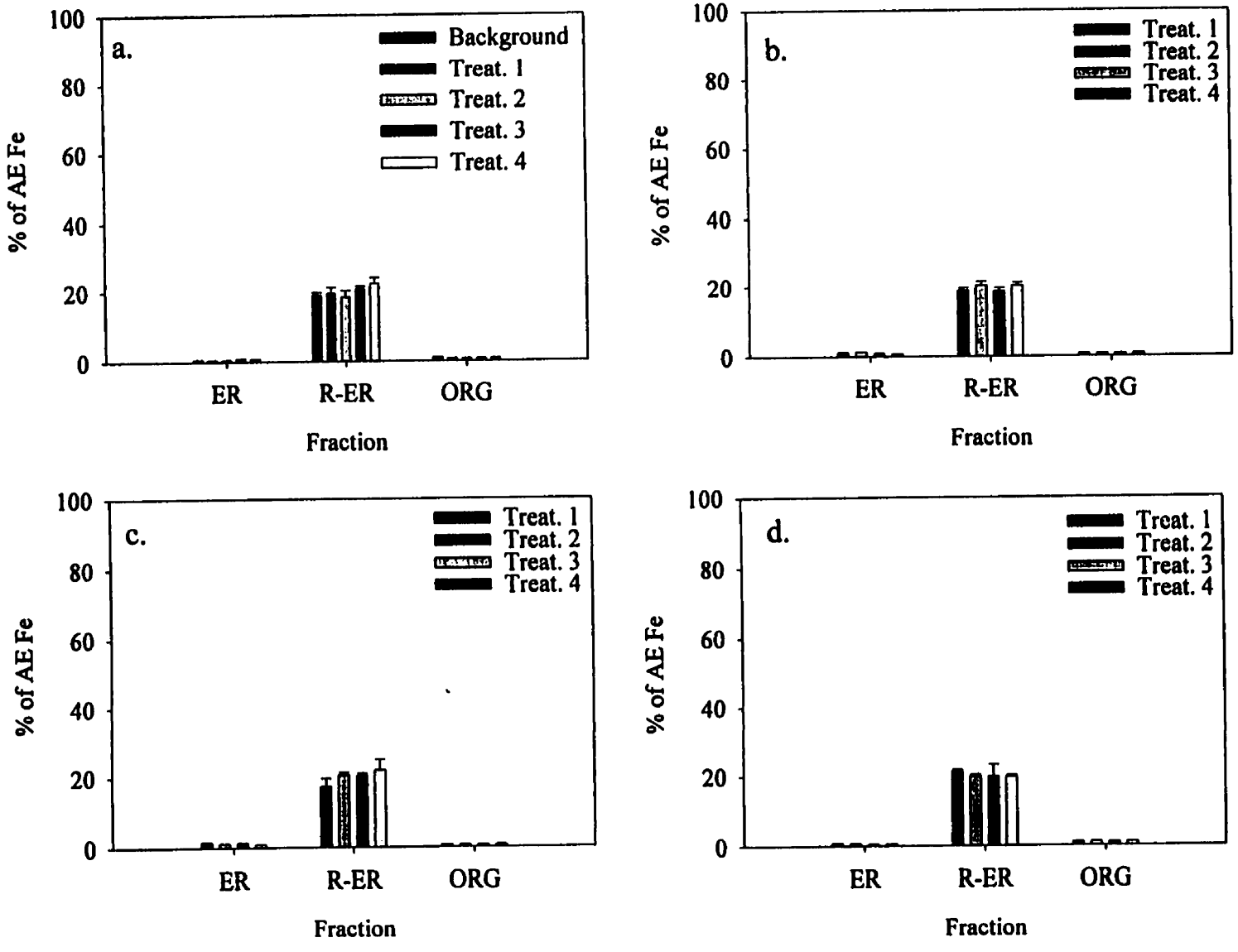
Appendix 10.6 Proportion of Cu associated with the ER, R-ER and ORG fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



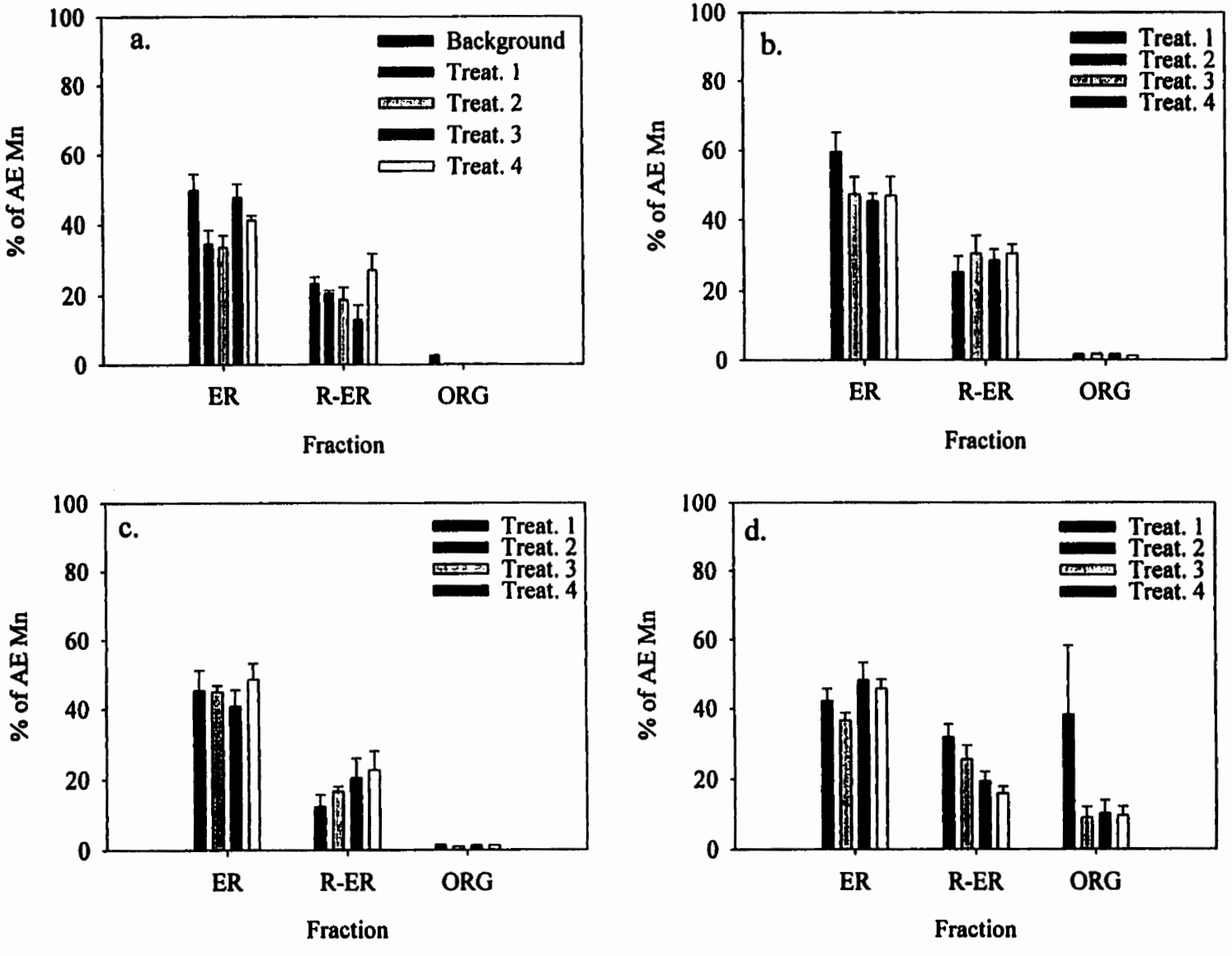
Appendix 10.7 Proportion of Zn associated with the ER, R-ER and ORG fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



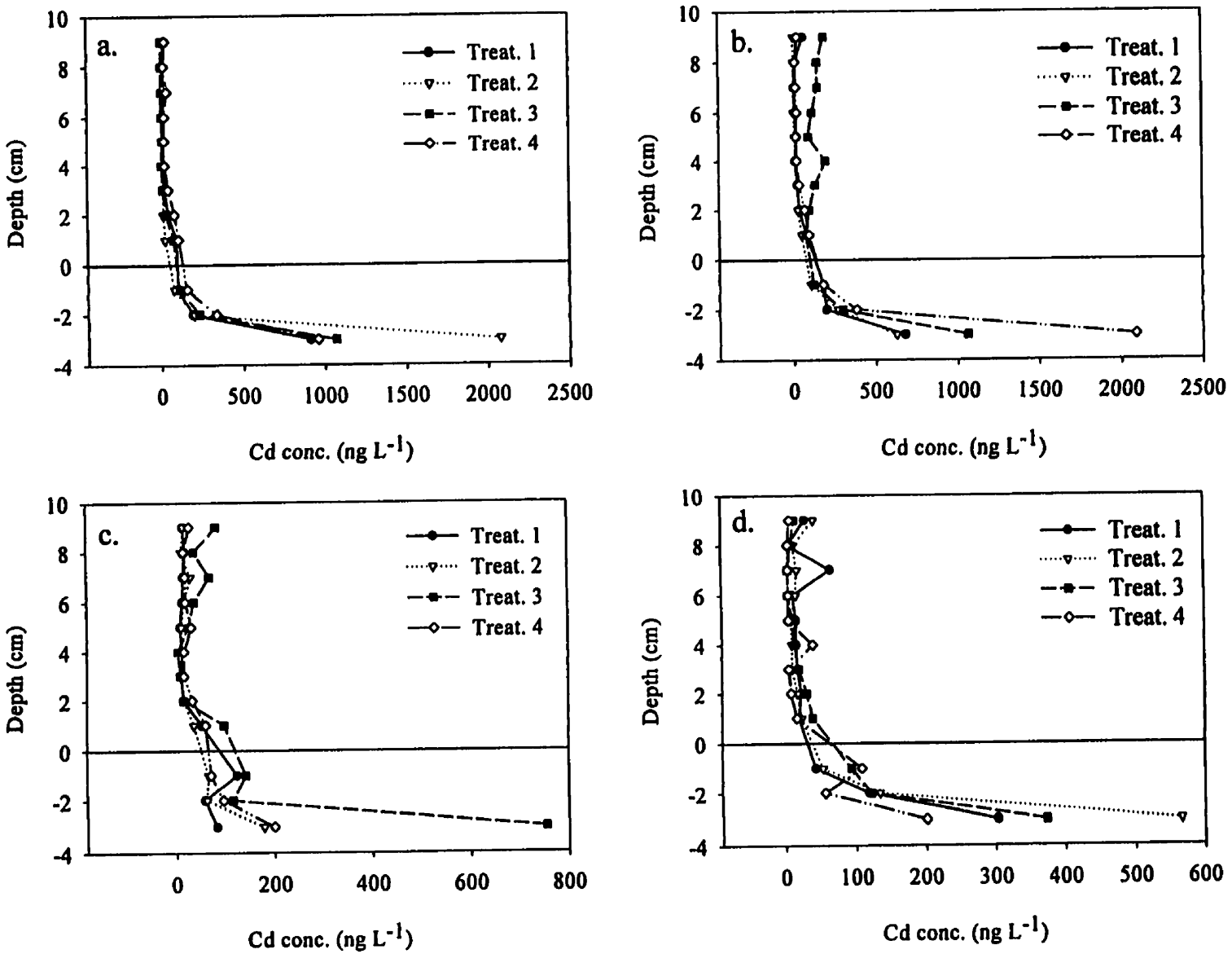
Appendix 10.8 Proportion of Pb associated with the ER, R-ER and ORG fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



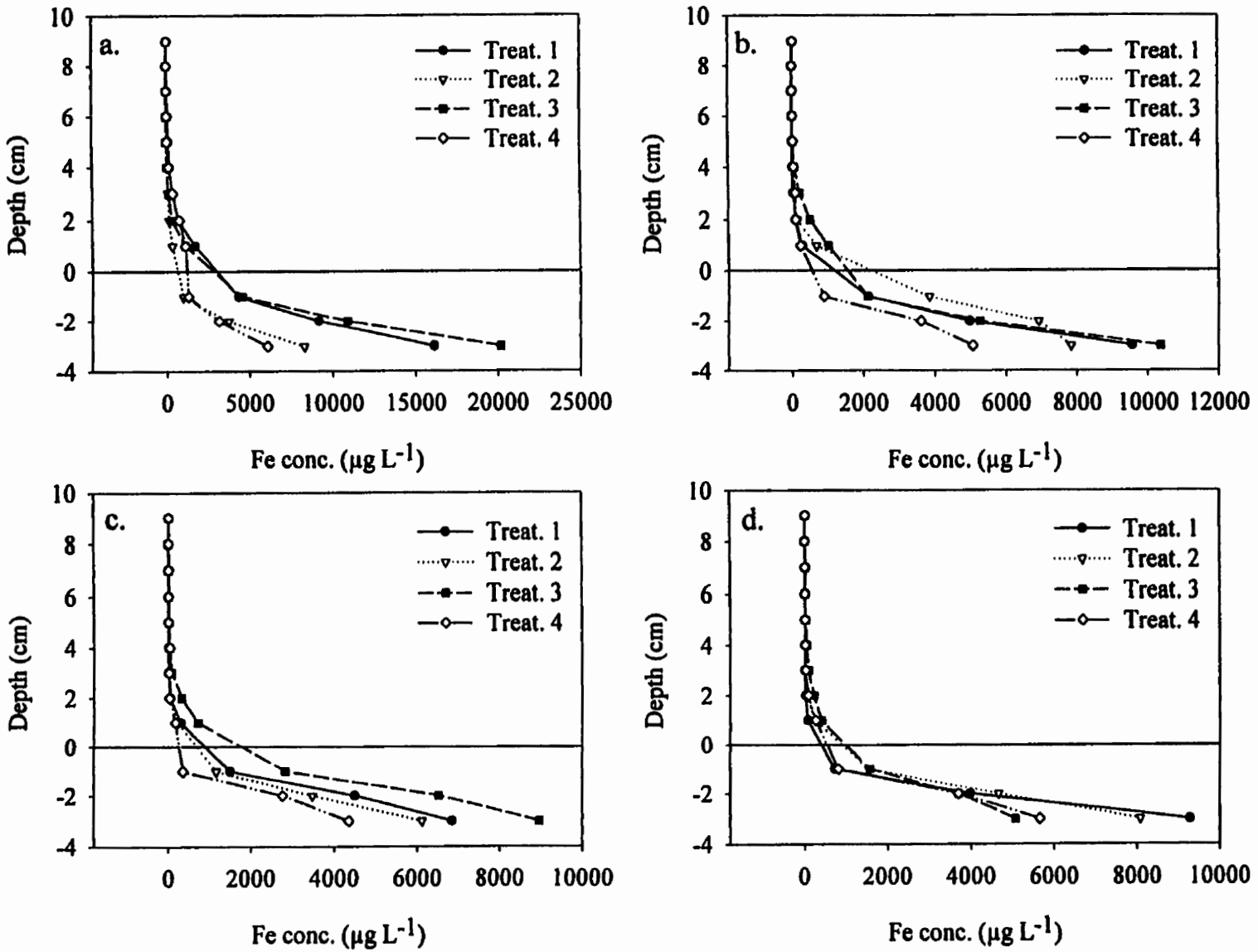
Appendix 10.9 Proportion of Fe associated with the ER, R-ER and ORG fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



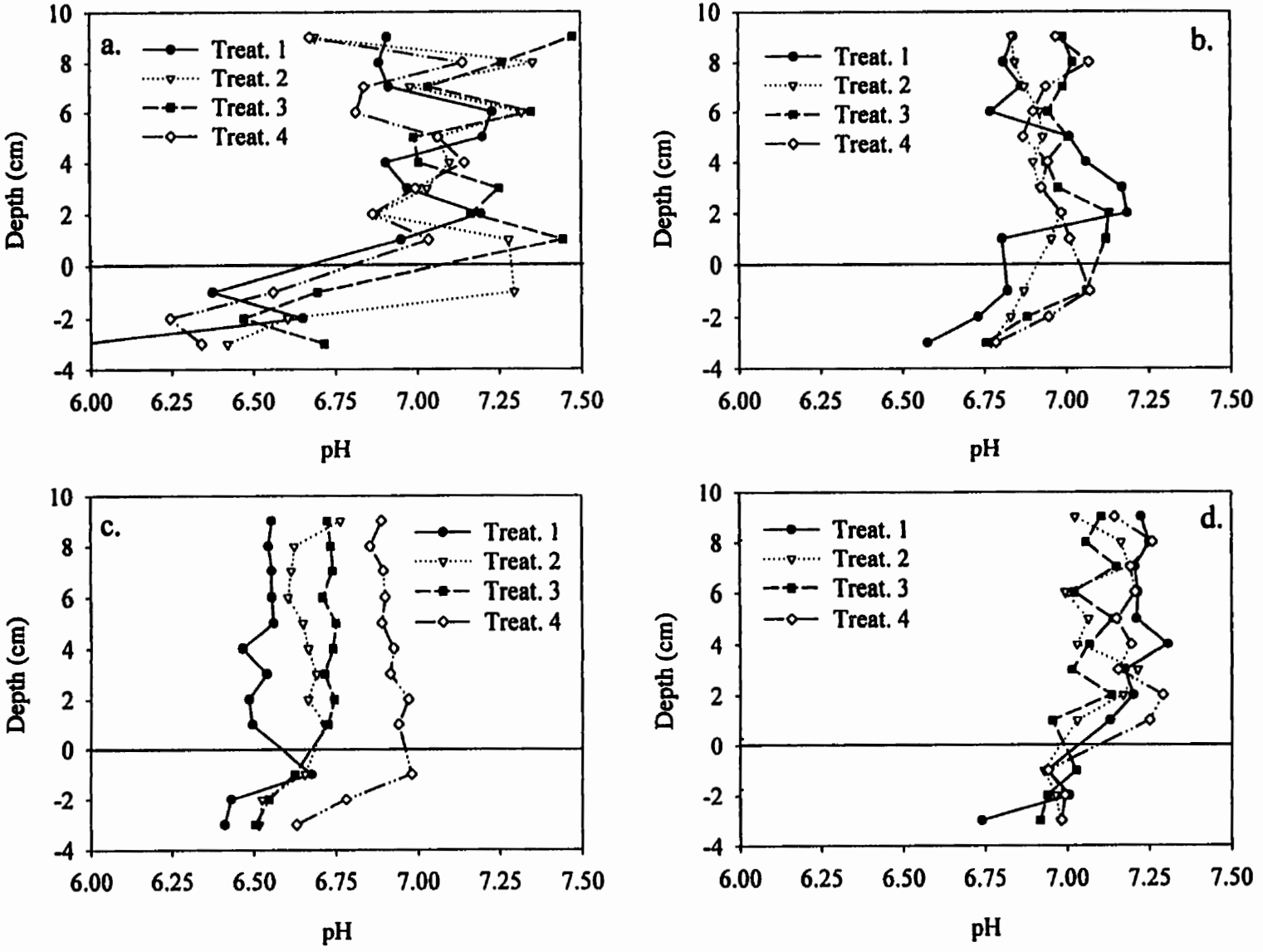
Appendix 10.10 Proportion of Mn associated with the ER, R-ER and ORG fractions. a. Background and post-spike samples. b. 2 weeks. c. 8 weeks. d. 10 weeks. Values are means (±SE, n=3).



Appendix 10.11 Porewater Cd (ng L^{-1}) profiles in the sediment and above the sediment-water interface. a. 4 weeks. b. 6 weeks. c. 8 weeks. d. 10 weeks. Note the sediment-water interface begins at 0 cm depth.

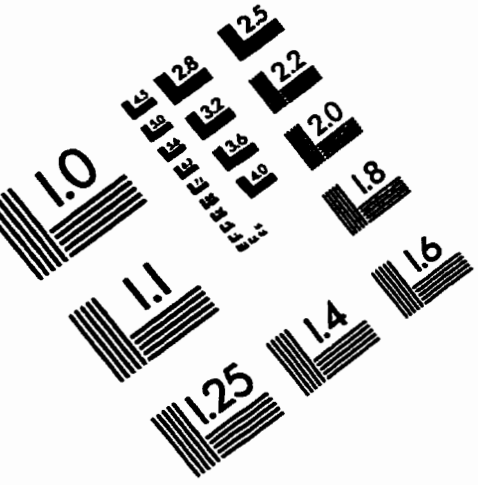


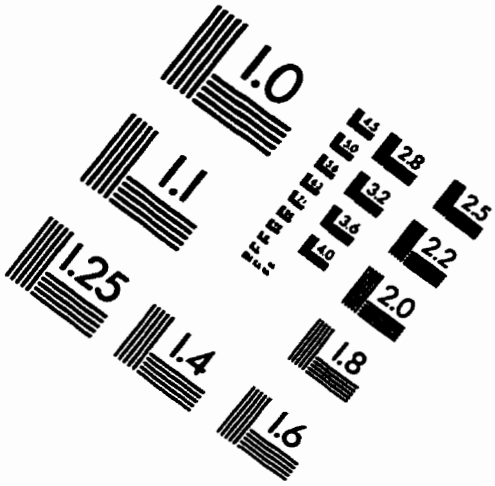
Appendix 10.12 Porewater Fe (µg L^{-1}) profiles in the sediment and above the sediment-water interface. a. 4 weeks. b. 6 weeks. c. 8 weeks. d. 10 weeks. Note the sediment-water interface begins at 0 cm depth.

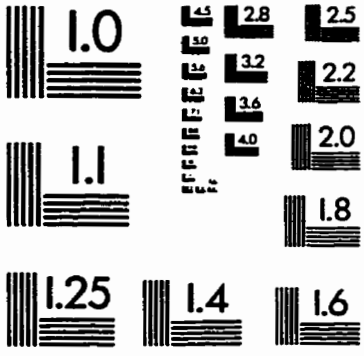


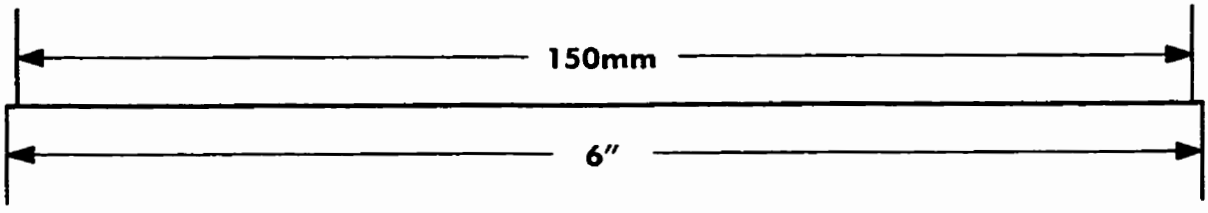
Appendix 10.13 Porewater pH profiles in the sediment and above the sediment-water interface. a. 4 weeks. b. 6 weeks. c. 8 weeks. d. 10 weeks. Note the sediment-water interface begins at 0 cm depth.

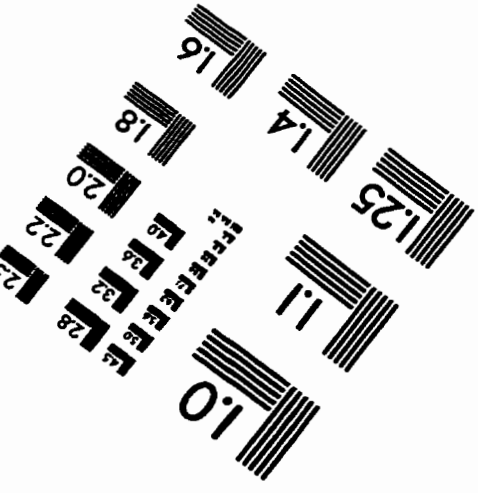
IMAGE EVALUATION
TEST TARGET (QA–3)

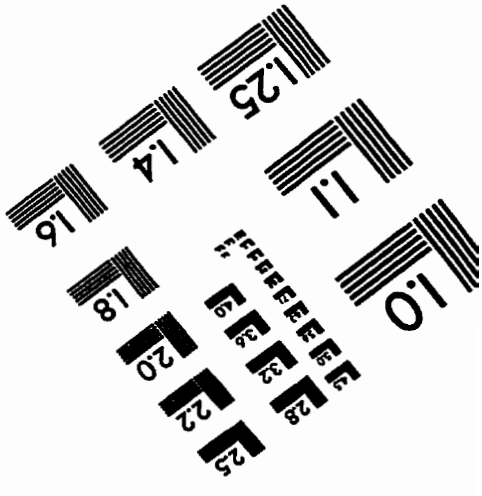












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989