THE RELATIVE SENSITIVITY OF BENTHIC INVERTEBRATES TO METALS

THE RELATIVE SENSITIVITY OF FOUR BENTHIC INVERTEBRATES TO SELECTED METALS IN SPIKED EXPOSURES AND APPLICATION TO CONTAMINATED FIELD SEDIMENT

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DANIELLE MILANI, B.SC.

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AUTHOR: Danielle Milani, B.Sc. (University of Guelph)

SUPERVISORS: Drs. T.B. Reynoldson and J. Kolasa

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ABSTRACT

The relative sensitivities of four benthic invertebrates (*Hyalella azteca*, *Chironomus riparius*, *Hexagenia* spp., and *Tubifex tubifex*) were determined separately for cadmium, copper, and nickel in 96-hour water-only and in spiked sediment exposures. Survival (LC25's and LC50's), growth and reproduction (IC25's) endpoints were compared amongst the four species. In the water-only tests, *H. azteca* is the most sensitive species to cadmium and nickel, with mean LC50's of 0.013 and 3.6 mg/L respectively, and *C. riparius* is the most sensitive species to copper, with a mean LC50 of 0.043 mg/L. In the spiked sediment exposures, *Hexagenia* spp. is most sensitive species to copper with a mean LC50 in sediment of 93 μg/g and a mean IC25 of 38 μg/g, and *H. azteca* is most sensitive species to cadmium and nickel, with mean LC50's of 33 and 67μg/g respectively and mean IC25's of 10 and 40 μg/g respectively. Overall, *T. tubifex* is the least sensitive species to all metals tested, and the number of young produced/adult is the most sensitive of the reproduction endpoints for *T. tubifex*. The relative sensitivities reveal that two endpoints, *Chironomus* and *Hexagenia* survival, can be used to possibly distinguish between cadmium, copper, and nickel metal toxicity. Species test responses in field-collected sediments from areas contaminated primarily by the above mentioned metals were compared to the determined sensitivities in order to establish the causative agent of toxicity.

Sediment toxicity was categorized first by comparing species responses to those established for a reference database. Responses in the field sediment support suspected toxicant in some cases, but not others. Multivariate analyses were used to assess sediment toxicity in the field sites based on the species responses, and these analyses reveal that the test endpoints respond to different environmental variables in ordination space. A comparison of test responses in the field sites to those in reference sites in ordination space reveals 13 of the 15 field-collected sites to be toxic or severely toxic.

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1. INTRODUCTION

Sediment toxicity tests are an important component in assessing the potential impact of sediment bound pollutants in the aquatic ecosystem, as they are a direct measure of functional response (Giesy and Hoke 1989). A weight of evidence approach using sediment toxicity test data in conjunction with chemical data and field-collected data are the basis by which the Canadian sediment assessment values for 8 metals (and 15 organic substances) were derived (Smith *et al.* 1996). While sediment toxicity tests can not determine cause and effect relationships without supporting studies (i.e. bioaccumulation, toxicity identification evaluation), they are nonetheless useful because they allow the assessment of potential toxicity of complex mixtures of chemicals and account for differential bioavailability in varying sediment types (Ankley *et al.* 1994).

Benthic invertebrates are considered the best indicators of sediment toxicity because of their intimate contact with sediment and interstitial water (ASTM 1993, USEPA 1994). Invertebrates spend all, or a large portion of their life cycle directly in the sediment and hence are continuously exposed to contaminants in the sediments. Since invertebrates are relatively sedentary, they are representative of local conditions (Canfield *et al.* 1994). However, no one species is adequate for detection of potential adverse effects of mixtures of contaminants (Giesy *et al.* 1990). Differences in species behaviour, lifestyle, and physiology may contribute to different sensitivities to contaminants among different groups of organisms. Differences in sensitivity amongst benthic organisms also tend to be contaminant-specific (Canfield *et al.* 1994, Kembel *et al.* 1994; Hickey and Martin 1995; Day *et al.* 1995a). While a test organism's response to a toxicant (or mixture of toxicants) can often be correlated with that of other organisms, this is not always the case (Giesy and Hoke 1989). The noted differences in species test response tend to be more pronounced in sediments of low to moderate toxicity (Day *et al.* 1995a). Therefore, the use of several species representing different sediment habitats, as well as the use of tests that measure different physiological endpoints are recommended for sediment to avoid arriving at incorrect conclusions about toxicity (Burton 1991; Day *et al.* 1995a; Suedel *et al.* 1996).

Previous research (Chapman 1986; Canfield *et al.* 1994; Reynoldson *et al.* 1995) has demonstrated the importance of integrating biological with chemical data. A study performed on Collingwood Harbour comparing metal concentrations in sediments with Ontario's chemical sediment quality criteria (Persaud *et al.* 1992) demonstrated the inability of the chemical criteria to determine the lack of impact (Reynoldson *et al.* 1995). For instance, sediments that exceeded the lowest effect concentrations for metals required biological data to determine whether the site was impacted. Also, sediment contaminant concentrations exceeded the severe effect concentrations at certain sites with no adverse biological responses evident in the benthic community structure or in the laboratory toxicity tests (Reynoldson *et al.* 1995).

Test Organisms

Four species of benthic invertebrates are used at the National Water Research Institute (NWRI), Burlington, Ontario, for the evaluation of sediment toxicity. They are the amphipod, *Hyalella azteca*; the midge, *Chironomus riparius*; the mayfly, *Hexagenia spp.*; and the oligochaete worm, *Tubifex tubifex*. These four species are representative of the major taxonomic groups occurring in the region. Oligochaetes, chironomids and amphipods comprise three of the four dominant taxonomic groups. Mayflies are also an important group of organisms intimately associated with the sediment (Giesy and Hoke 1989), and have received considerable attention due to their disappearance and subsequent recovery in the western basin of Lake Erie (Reynoldson *et al.* 1989,1993; Krieger *et al.* 1996). All four species are important as food sources for juvenile and adult fish, predacious aquatic insects, amphibians, or birds (Pennak 1953). They are potentially exposed to contaminants from the interstitial water, overlying water and sediment (Pennak 1953), are responsive to environmental changes in sediment, and as such, are useful in the monitoring sediment contamination (Schloesser *et al.* 1995). These four species, in conjunction, have been used in the evaluation of toxicity of single chemicals and mixtures of chemicals in contaminated sediments at NWRI (Reynoldson *et al.* 1994, Reynoldson and Day 1994, 1998a; Zeman *et al.* 1995; Day *et al.* 1995a, 1995b, 1998; Cheam *et al.* 1999). Additionally, the relationship between the functional responses (survival, growth and reproduction) of the four species in sediment bioassays along with benthic community structure data and key environmental variables, methods derived from the sediment quality triad approach (Chapman 1986), have been recently used to develop quantitative guidelines for near shore sites of the Great Lakes (Reynoldson *et al.* 1995,1997; Reynoldson and Day 1998b). This involved the compilation of sediment bioassay data accumulated from sites that represented the natural variation in physico-chemical characteristics of 'clean' lake sediments and the associated behaviour of the four test organisms. A range of functional test response was established from the reference data for each species defined by three categories of toxicity: non-toxic, potentially toxic, and toxic (see below). This establishment of a reference state, or the *reference condition concept* (Reynoldson and Day 1998b), is useful as it describes the normal response range for each species. Thus, an organism's response to anthropogenic stress can be differentiated from natural variability (Reynoldson and Day 1998b). This offers an advantage over selecting standard control sites (upstream-downstream), which may not represent the condition at test sites and often do not have the same confounding factors (i.e. sediment geochemistry, particle size distribution) as the test sites (Maund *et al.* 1999).

Metal sensitivity

In the above-mentioned NWRI studies (Reynoldson *et al.* 1994, Reynoldson and Day 1994, 1998a; Zeman *et al.* 1995; Day *et al.* 1995a, 1995b, 1998; Cheam *et al.* 1999) differential responses were exhibited by the four benthic species. The variables (contaminants) that modified the test responses, however, were not positively identified. Knowledge of the four species sensitivity to different compounds (i.e. organic and metal contaminants) could aid in evaluating the source(s) of toxicity in sediments. Determining sensitivities to certain metals is thus an important first step. Metals (essential and non-essential) have adverse effects on benthic invertebrates when present in sufficient concentration, or when conditions are conducive to bioaccumulation. These adverse effects are generally thought to occur through the damage to plasma membranes and disruption or interference of metabolic pathways (Depledge *et al.* 1998). Effects exerted by metal toxicity can be lethal (death) or sublethal. Sublethal responses may include avoidance (McMurtry 1984; Wentzel *et al.* 1977a,b), impaired/decreased growth and reproduction (Powlesland and George 1986; Pascoe *et al.* 1989), decreased oxygen consumption (gill damage) or prevention of gas exchange leading to respiration decrease (Brković-Popović and Popović 1977b; Whitley 1967; Hodson *et al.* 1979), structural deformities, delayed development and emergence in aquatic insects (Diggins and Stewart 1998; Wentzel *et al.* 1978; Kosalwat and Knight

1987a; Pascoe *et al.* 1989), ion balance/regulation problems (Gerhardt 1995), and body deterioration/fragmentation (Hodson *et al.* 1979). These sublethal responses may affect the overall fitness of a population and may ultimately lead to lethality (Heinz 1989).

There are many complex factors that may affect species sensitivity to metals. Theoretically, differences in species metal sensitivities may be due to differences in uptake routes, regulatory capabilities, storage and excretion, detoxification capabilities, gut passage time, and differences in internal distribution or localization of the metal (Hare *et al.* 1991; Krantzberg and Stokes 1989,1990; Marr *et al.* 1995). Biotic factors (i.e. sex, molting, body weight, behaviour), abiotic factors (i.e. free aqueous metal ion, pH, dissolved organic matter), sediment geochemistry (i.e. redox, proportion of different solid fractions) (Luoma 1983,1989; Tessier *et al.* 1984; Tessier and Campbell 1987; Wang 1987; Kersten and Forstner 1987; Campbell *et al.* 1988; Van Hattum *et al.* 1991), and metal binding intensities (Luoma and Jenne 1977; Tessier and Campbell 1987; Luoma 1989; Timmermans 1993) may also affect toxicity (or metal accumulation).

Relative sensitivity in metal-spiked exposures

Cadmium, copper, and nickel represent metals of concern in the Canadian environment as they are important products or by-products in the mining and smelting industries, and may enter the environment either directly or indirectly through atmospheric deposition. Previous studies comparing the relative sensitivities of benthic invertebrates to these metals (and other contaminants) determined lethal responses (LC50's) in both water-only tests and in contaminated sediments (Giesy *et al.* 1990; West *et al.* 1993; Collyard *et al.* 1994; Hickey and Martin 1995; Ingersoll *et al.* 1995; Phipps *et al.* 1995; Suedel *et al.* 1996). Sublethal endpoints may be better at identifying marginally contaminated sediments (Ingersoll *et al.* 1998); can reveal the mechanism of action of a toxicant; and can also serve as a potential warning sign (Heinz 1989). The inhibition concentration (IC), the point estimate of a toxicant causing a given percent change in a specific response (i.e. growth) compared to a control (Norberg-King 1993), is recommended by Environment Canada as the statistical endpoint for sediment tests with *H. azteca* and *C. riparius* (McLeay and Day 1997a,b). No studies have compared the relative sensitivities (using sublethal and lethal responses) of these four organisms employed by Environment Canada in their evaluation of sediment toxicity, nor has there been an attempt to extrapolate results to field-collected sediment in an attempt to determine possible causative agent of toxicity. Since exposure routes may differ in waterborne versus sediment exposures, and since sediments can alter the bioavailability of contaminants, measurement of species sensitivities in water-only tests does not seem adequate. Alternatively, a natural 'clean' sediment spiked with the contaminant can be used to determine relative sensitivities and would be more representative of conditions in nature.

The determination of LC50's in the overlying water and pore water fractions (in addition to the bulk sediment fraction) in spiked-sediment exposures is useful as it allows the comparison to the LC50's from water-only exposures. In addition, determination of LC25's in the spiked exposures permits the comparison to the IC25's, thus allowing survival and growth endpoints to be compared.

Application of relative sensitivities to field-contaminated sediments

Knowledge of the relative sensitivities of the four species to different metals could aid in determining the causative agent(s) of toxicity in metal-contaminated field sediments. This can be done by comparing established sensitivities (LC25's/LC50's) of the four species in metal-spiked sediment exposures to species test responses in field-contaminated sediments (see below). Thus, the comparison of the species sensitivities can be used as a relative measure to interpret metal toxicity in a fairly quick and simple manner.

Field-collected sediments used in this study were contaminated primarily by the metals examined in the metal-spiked exposures to compare species responses in these sediments with the established sensitivities from the laboratory-spiked sediments. The three regions chosen for the field evaluation represented areas impacted by metals due to either shipbuilding or mining industries (Couillard *et al.* 1993; Reynoldson and Day 1994, 1998a; Ecological Services Group 1996). The areas were thought to be impacted by cadmium, nickel, or copper. Collingwood Harbour, Ontario, a historical shipping port, has received metal contamination from the ship building industry. Although the industry closed in 1986, sediments, notably in the east and west boat slips, contain elevated metals (Reynoldson and Day 1994,1998b). The second area, Levack (near Sudbury, Ontario), is one of the leading nickel producers in the world and has been the site of nickel mining for over a hundred years. Widespread nickel (and copper) contamination of surrounding aquatic areas has occurred directly as well as indirectly through atmospheric deposition (Stokes 1981). A survey on the Onaping River (Ecological Services Group 1996) revealed elevated nickel and copper metals downstream of mine effluent discharge relative to an upstream reference area. The third area, lakes near the Rouyn-Noranda, Québec, exhibit a spatial gradient of cadmium concentrations with varying distances from major mining and smelting operations in this region (Couillard *et al.* 1993). Cadmium accumulation has been reported in the benthos from various lakes in this region (Tessier *et al.* 1984; Hare *et al.* 1989,1991; Couillard *et al.* 1993).

The use of multivariate techniques

Multivariate techniques such as ordination are well-established in ecology (i.e. plant community studies), and their use in ecotoxicology is also becoming more frequent and recommended (Maund *et al.* 1999; Sparks *et al.* 1999). Much of this work involved the examination of benthic community structure (i.e. species presence and abundance) in which multivariate methods allowed a greater understanding of variables involved in each system and the relationship with communities (Jackson 1993). The use of multivariate techniques in assessing the response of test species in sediment toxicity tests and the relationship to environmental data can also be very useful (Reynoldson 1994; Reynoldson and Day 1998b). For instance, information on the four species responses can be combined to provide a more comprehensive analysis of the sediment, which in turn would better assist management decisions when determining which areas are impacted and to what degree, or whether remediation was effective (Reynoldson and Day 1998b). Multivariate methods should also discriminate among the relative toxicity response of the organism.

The approach of using test organism response in sediment toxicity tests in relation to environmental attributes is currently not done in ecotoxicology studies, and as such, will be explored as a tool to assess sediment toxicity from field-collected sediments and also to assess the extent of toxicity from the field-collected sediment with respect to reference sites.

Study Objectives

In summary, the goals of this study are:

1. Determine the relative sensitivities of *H. azteca*, *C. riparius*, *Hexagenia spp.*, and *T. tubifex* to cadmium, copper, and nickel.
2. Compare the species test responses in field-collected sediments contaminated with cadmium, copper, or nickel to normal response ranges in 'clean' sites established for the Great Lakes region to determine level of toxicity at each site.

3. Use the four species relative sensitivities as a diagnostic tool for interpreting whether metal of interest is possibly eliciting test responses in the field-contaminated sediments.
4. Examine the interactions between the metals of interest in the field-collected sediments and the modifying sediment attributes using multivariate methods.

2. MATERIALS AND METHODS

2.1 Culture Methods

All organisms were maintained at the Ecotoxicology Laboratory at NWRI in environmental chambers. Organisms were cultured at 23°C ± 1 °C, under a photoperiod of 16 hours of light and 8 hours of dark (16L: 8D), and under an illumination of 500-1000 lux, with the exception of *T. tubifex* which was cultured in the dark. All cultures were aerated by means of aquarium pumps and air stones (with the exception of *H. azteca* brood jars, which were not aerated).

Water used for culturing was the City of Burlington tap water (Lake Ontario). Prior to use, the water was charcoal filtered and aerated for a minimum of three days. Water characteristics included: conductivity 273 – 347 μS/cm; pH 7.5 - 8.5; hardness 120 - 140 mg/L; alkalinity 75 - 100 mg/L; and chloride ion 22 - 27 mg/L.

Sediment was used as a substrate in the rearing of *Hexagenia* spp. and culturing of *T. tubifex*. This was a marsh sediment acquired from Long Point, Lake Erie, composed on average of 70.33% silt, 29.13% clay, 0.54% sand, and 8.1% organic carbon. Sediment was collected in the spring and fall of each year (1997 & 1998) with a mini-ponar sampler and stored at 4 °C in the dark. Prior to use, the sediment was wet sieved through a 250-μm mesh screen into 10-L plastic buckets, and the residue discarded. The sediment was allowed to settle in the buckets for a minimum of 24 hours, after which the water was decanted. A summary of all culture methods is shown in Table 2.1.

Table 2.1 Summary of culture methods.

Culture Conditions	***H. azteca***	***C. riparius***	***Hexagenia* spp.**	***T. tubifex***
Container	2 L wide mouth jars	20 L aquaria	20 L aquaria	Plexiglas tanks (20 cm X 20 cm X 20 cm)
Substrate	Medicinal Gauze	Natural aquarium gravel	Long Point marsh sediment	Long Point marsh sediment
Starting no. of animals/tank or jar	25 – 30 adults	3 hatched egg masses	300 newly hatched nymphs	200 full cocoons
Illumination	500-1000 lux	500-1000 lux	500-1000 lux	None
Photoperiod	16L:8D	16L:8D	16L:8D	None
Culture period	Weekly separation of young from adults	Until adult emergence (~ three weeks)	6 weeks, or until nymphs are between 5 - 8 mg wet weight	8 weeks, or to sexual maturity (gonads visible)
Feeding	5 mg fish flakes thrice weekly	fish flakes *ad libitum*	4 mL prepared diet once weekly	None

2.1.1 *Hyalella azteca*

The culturing of *H. azteca* is based on methods described in Borgmann *et al.* (1989), and Reynoldson *et al.* (1998). Maintenance stock cultures of *H. azteca* were kept in 20-L aquaria with 8-L culture water. Medicinal gauze (pre-soaked for 24 hours in distilled water) was used as a substrate. To provide the young of a known age, adult amphipods (sexually mature if possible) were maintained separately in 2-L wide mouth jars (brood jars) containing 1 L of culture water and one 2.5 X 2.5 gauze strip. The brood culture consisted of 20-25 brood jars, each containing 25-30 adults, which produced from 300 to 1200 young per week.

Once weekly, the young were separated from the adults by pouring the contents of each brood jar through 500 μm and 250-μm nitex sieves (5 cm in diameter) sequentially. The adults were retained on the 500-μm sieve, while the young passed through the 500-μm sieve and were retained on the 250-μm sieve. Amphipods from both sieves were washed separately into petri dishes for enumeration. The number of adults and mating pairs were counted and returned to the jar containing 1-L fresh culture water. The young were counted and kept in a separate jar with 1 L of culture water until used in tests. Maintenance cultures were fed Nutrafin® fish flakes (crushed into a fine powder) twice weekly *ad libitum*. Brood jars were fed 5-mg crushed Nutrafin® fish flakes thrice weekly on non-consecutive days.

2.1.2 Chironomus riparius

The culture of *C. riparius* is described in Day *et al.* (1994), and Reynoldson *et al.* (1998). Chironomids were maintained in 20 L aquaria containing 2 cm deep natural aquarium gravel and 8 L culture water.

Each culture was initiated with three hatched egg masses. Constructed Plexiglas additions (40 cm X 19.5-cm X 20.5 cm) were placed onto the lip of the aquaria prior to the emergence of the organisms (at the fourth instar stage), to prevent the escape of emerged adults and to allow the adults further room to mate. Under the culture regime described above (temperature, light intensity, and photoperiod), adults deposited their eggs in approximately three to four weeks from culture initiation. Deposited egg masses were removed daily with a net and tweezers and examined under a dissecting microscope. Hatched egg masses (organisms are hatched but still attached to the egg mass at this point) were separated and kept aside for testing purposes, or used to initiate another culture. Cultures were fed crushed Nutrafin® fish flakes *ad libitum*.

2.1.3 Hexagenia spp.

Eggs of *Hexagenia* spp. were collected from gravid females in Windsor (Lake St. Clair), Ontario, in July of 1996 and 1997, according to procedures described in Hanes and Ciborowski (1992). Upon arrival at the laboratory, the eggs were stored at 4 °C to delay hatching. *Hexagenia* were reared according to procedures described in Bedard *et al.* (1992) and Reynoldson *et al.* (1998).

Mayflies were reared in 20-L aquaria containing a 2-cm layer of culture sediment and 8-L culture water. Once weekly, approximately 2 mL of eggs were removed from the cold room and brought to 23°C ± 1 °C in 50 mL Petri dishes containing culture water. Once hatched, mayfly nymphs were transferred to culture tanks (300 per tank). Nymphs were reared for approximately 6 weeks until they reached the size used for testing 5 – 8 mg-wet weight/nymph). Cultures were fed 4 mL weekly of a prepared diet, consisting of 4 g crushed Nutrafin® fish flakes, 3 g Cerophyll™, and 3 g brewers yeast homogenized in 100 mL distilled water.

2.1.4 Tubifex tubifex

The culture of *T. tubifex* is described in Reynoldson *et al.* (1991) and Reynoldson *et al.* (1998). Approximately 2 L of culture sediment and 4-L culture water were added to a Plexiglas tank (20 cm X 20 cm X 20 cm container with fitted lid). A new tank was initiated with the addition of 200 full cocoons. Cocoons were removed from the sediment by sieving through a 500-μm mesh screen. With the aid of a dissecting microscope, 200 full cocoons were counted and added to a new culture tank. Sexually mature worms were set aside for testing purposes. This process was repeated weekly, insuring the availability of *T. tubifex* each week.

2.2 Sediment Toxicity Tests

Sediment toxicity test methods were based on procedures described in Borgmann and Munawar (1989), Borgmann *et al.* (1989), Kranzberg (1990), Reynoldson *et al.* (1991), and Reynoldson *et al.* (1998). All tests passed an acceptability criteria based on percent control survival before being included in a data set, i.e. ≥ 80% for *H. azteca* and ≥70% for *C. riparius* (USEPA, 1994; ASTM, 1995); ≥80% for *Hexagenia* spp., and ≥75% for *T. tubifex* (Reynoldson *et al.* 1998).

The water used for experiments was the same source as used in culturing. Water chemistry variables (pH, dissolved oxygen (mg/L), conductivity (μS/cm), temperature (°C), and total ammonia (mg/L)) were measured for each test in each replicate test beaker on day 0 (start of test) and at completion of the test (day 10, day 21, or day 28). Total ammonia was measured in each treatment by taking a sample from each beaker. During this time all test beakers were aerated, and water loss due to evaporation was replaced daily with de-ionized water. Tests were run under static conditions in environmental chambers at 23°C ±1 °C, under a photoperiod of 16L: 8D and an illumination of 500 - 1000 lux, with the exception of *T. tubifex* test which was run in the dark. Test beakers were gently aerated to maintain between 50 - 100 % saturation. Air delivery was by means of aquarium air pumps and capillary tubes (0.5 mm), (*H. azteca, C. riparius and T. tubifex*), or Pasteur pipettes (*Hexagenia spp.*). To prevent evaporation, each test beaker was fitted with a lid with a central hole for aeration purposes.

Tests were conducted in 250-mL glass beakers, with 75-mL sediment and 113-mL overlying water for *H. azteca* and *C. riparius*, and 100-mL sediment and 150-mL overlying water for *T. tubifex*. Tests with *Hexagenia* spp. were conducted in 1-L glass jars with 300-mL sediment and 450 mL overlying water. For the spiked sediment exposures, each test consisted of an un-spiked control, a minimum of five test concentrations with two to five replicates per concentration, and an additional beaker for chemical analysis. Each complete range of spiked concentrations was repeated a minimum of three times for each metal and for each species. Range finding tests were conducted initially to determine the appropriate concentrations for each species for each metal. For the toxicity tests conducted with the field-collected sediment, five replicate beakers per sediment were set up with an additional beaker for chemical analysis. A summary of all test conditions is given in Table 2.2.

Table 2.2 Summary of test conditions for sediment toxicity tests.

Test Conditions	***H. azteca***	***C. riparius***	***Hexagenia* spp.**	***T. tubifex***
Test Duration	28 days	10 days	21 days	28 days
Feeding	8 mg fish flakes twice weekly	8 mg fish flakes 3X throughout test	50 mg prepared diet once weekly	80 mg fish flakes once, prior to addition of organisms
Age/size of organism used	2 – 10 days	1st instar	5 - 8 mg wet weight	sexually mature
No. of organisms per test beaker	15	15	10	4
Photoperiod	16L:8D	16L:8D	16L:8D	none
Number of replicates per concentration/ sediment	2-5/5	2-5/5	2-5/5	2-5/5
Endpoints measured	% survival; growth (mg dry weight)	% survival; growth (mg dry weight)	% survival; growth (mg dry weight)	% adult survival; # young/adult; #cocoons/adult; % cocoons hatched

2.2.1 *Hyalella azteca 28-day growth and survival test*

The *H. azteca* test was conducted for 28 days using 2 -10 day old organisms. Amphipods were selected at random and added to small plastic weight boats with a pipette (5¾-mm) until 15 individuals per boat was achieved. The contents of weigh boats were added to replicate beakers. Floating animals, if present, were gently pushed below the water surface. Each test beaker was fed 8 mg Nutrafin® fish flakes twice weekly on non-consecutive days. On day 28, the contents of each beaker were rinsed through a 250-μm screen and the surviving amphipods counted. Amphipods were then dried at 60 °C for 24 hours and dry weights recorded.

2.2.2 Chironomus riparius 10-day growth and survival test

The *C. riparius* test was conducted for 10 days using first instar organisms. Fifteen chironomids were added to the test beaker randomly by a pipette (5¾-mm). Each test beaker was fed 8-mg Nutrafin® fish flakes three times throughout the test on non-consecutive days. On day 10, the contents of each beaker were wet sieved through a 250-μm screen and the surviving chironomids counted. Chironomids were then dried at 60 °C for 24 hours and dry weights recorded.

2.2.3 Hexagenia spp. 21-day growth and survival test

The *Hexagenia* spp. test was conducted for 21 days using nymphs weighing between 5 - 8 mg wet weight/nymph. Mayflies were removed from the sediment by sieving through a 500-μm sieve, and were subsequently rinsed into a two-inch deep tray for sorting. Ten mayflies were randomly added to large plastic weight boats (one boat for each test replicate) containing culture water.

Initial weights of the mayflies were measured as a group of ten and added to the test jars. Each test jar was fed 50 mg of a prepared diet (Cerophyll™, Nutrafin®, and brewers yeast) once weekly. On day 21, the contents of each jar were wet sieved through a 500-µm screen and surviving mayfly nymphs counted. Nymphs were then dried at 60 °C for 24 hours and dry weights recorded.

Initial wet weights were converted to dry weights with the following conversion: Initial dry weight = (Initial wet weight + 1.15)/7.35 (Reynoldson *et al.* 1998). Growth was determined by subtracting the initial dry weight from the final dry weight.

2.2.4 Tubifex tubifex 28-day reproduction and survival test

The *T. tubifex* test was conducted for 28 days using sexually mature worms (gonads visible). Each test beaker was supplemented with 80-mg crushed Nutrafin® fish flakes prior to the addition of the worms. Adult worms were removed from the sediment by sieving through a 500-µm screen and then rinsed into petri dishes for sorting. Sexually mature worms were randomly added to small plastic weight boats with a probe until four per boat was achieved. A magnifier was used to ensure that there were no immature worms attached to the adults. The contents of each boat were added to the replicate beakers. On day 28, the contents of each beaker were rinsed through a 500-µm and 250-µm sieve sequentially. The number of surviving adults, full cocoons, empty cocoons, and large immature worms were retained on the top sieve, and the number of small immature worms were retained on the bottom sieve. The contents of each sieve were rinsed into separate gridded petri dishes for enumeration with a dissecting microscope. Endpoints measured were percent adult survival, number of young produced per adult, number of cocoons produced per adult, and the percent of cocoons that hatched.

2.3 Sediment Characterization

Sediment characterization was performed by the Sedimentology Laboratory, NWRI, Burlington, ON. Sediment used in the spiking tests and the field-collected sediments was analyzed for particle size and total organic carbon content. Particle size analysis was done following the procedure of Duncan and LaHaie (1979). A homogenized sample of the sediment was dispersed in sodium metaphosphate and mixed for 15 minutes. The sample was then sieved through a 63-μm screen. The residue on the sieve was dried, and recorded as percent sand and gravel. The suspension that passed through the sieve was analyzed for percent silt and clay utilizing a sedigraph analyzer.

Total organic carbon in the sediments was determined by drying a homogenized sediment sample for a minimum of two hours, then burning 0.1 g of the dried sample for 250 seconds at 500°C. The percentage of organic carbon was determined by dividing the final weight of the sample by the initial weight (0.1g) X 100.

2.4 Water-Only/Sediment Exposures

2.4.1 *96-hour water-only exposures*

Water-only tests were conducted under static conditions for 96-hours in 250-mL glass beakers. A substrate was used in each test with the exception of the *T. tubifex* tests. Substrates employed were: 2.5 x 2.5 nitex screens for *H. azteca* (Borgmann *et al.* 1989), a monolayer silica sand for *C. riparius* (ASTM 1993), and constructed glass tubes for *Hexagenia* spp. (Henry *et al.* 1986). Test beakers were supplemented with 4-mg crushed Nutrafin® fish flakes on day 0 and day 2. All tests passed an acceptability criterion of ≥90% control survival before being included in a data set.

Each of the metal stock solutions was prepared by dissolving reagent grade cadmium (as $CdCl_2{\cdot}2½H_2O$), nickel (as $NiCl_2{\cdot}6H_2O$), or copper (as $CuCl_22{\cdot}2H_2O$) in milli-Q water. To achieve the desired concentration of metal in each dilution series, the appropriate aliquot of metal stock (0 - 100 mL) was added to a graduated cylinder, brought to 200 mL with the addition of culture water, and poured into test beakers. Water chemistry variables (pH, dissolved oxygen (mg/L), conductivity (µS/cm) and temperature (°C)) were measured on day 0 (start of test) and at the completion of the test. Test beakers were not aerated during the test, and were loosely covered with a plastic liner to minimize evaporation. Tests were run at 23°C ± 1 °C in environmental chambers. The

H. azteca and *C. riparius* tests were run under a photoperiod of 16L: 8D, and under an illumination of 500-1000 lux. The *T. tubifex* and *Hexagenia* spp. tests were run in the dark. Each test consisted of an un-spiked control (100% culture water) plus five to six spiked concentrations, with one to six replicates per concentration. Each complete range of spiked concentrations was repeated a minimum of three times for each metal and for each species. A summary of test conditions is given in Table 2.3 and nominal spiking concentrations are given in Table 2.4.

Table 2.3 Summary of test conditions for 96-hour water-only exposures.

Test Conditions	***H. azteca***	***C. riparius***	***Hexagenia* spp.**	***T. tubifex***
Test Duration	96 hours	96 hours	96 hours	96 hours
Feeding	4 mg fish flakes days 0 & 2	4 mg fish flakes days 0 & 2	4 mg fish flakes days 0 & 2	4 mg fish flakes days 0 & 2
Age/Size of organism used	2 -10 days	1st instar	5-8 mg wet weight	Sexually mature
No. of organisms per test beaker	10	10	5	4
Photoperiod	16L:8D	16L:8D	None	none
Substrate Used	nitex mesh	Monolayer silica sand	Constructed glass tubes	none

Table 2.4 Nominal concentrations of spiked metal (mg metal/L) in 96-hour water-only exposures.

Metal	***H. azteca***	***C. riparius***	***Hexagenia* spp.**	***T. tubifex***
Cadmium	0, 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05	0, 0.001, 0.0025, 0.005, 0.01, 0.025, 0.05	0, 0.25, 0.5, 1, 5, 10, 50	0, 0.1, 0.5, 0.75, 1, 2.5, 5
Copper	0, 0.025, 0.05, 0.1, 0.25, 0.5, 1	0, 0.025, 0.05, 0.1, 0.5, 1, 5	0, 0.01, 0.025, 0.05, 0.1, 0.5, 1	0, 0.05, 0.1, 0.25, 0.5, 1, 5
Nickel	0, 0.5, 1, 2.5, 5, 10, 25	0, 0.1, 0.5, 1, 5, 10, 50	0, 0.5, 1, 5, 10, 50, 100	0, 5, 10, 25, 50, 100

2.4.2 Spiking of sediment

The sediment used in the metal-spiked tests was collected from a reference site (303) located close to Long Point in Lake Erie (42°33'54" N, 80°02'28" W). Sediment was pre-sieved using a 250 μm mesh into 10-L plastic buckets to remove indigenous species. Spiking procedures were those described in Milani *et al.* (1996). Nominal concentrations are shown in Table 2.5. Batches of wet sediment (1 to 1.5 L) were spiked with the appropriate aliquot of metal and placed in 2-L square sided glass containers. The sediment was then homogenized by placing on a side-to-side shaker for 90 minutes at 175 agitations per minute. The sediment was dispensed into test beakers and the overlying culture water added in a 1.5:1 ratio of overlying water to sediment (volumes previously stated in section 2.2). Test beakers were equilibrated for two weeks with a one-week aeration time prior to commencement of tests.

Table 2.5 Nominal concentrations of metal (μg metal/g) in spiked-sediment exposures.

Metal	***H. azteca***	***C. riparius***	***Hexagenia* spp.**	***T. tubifex***
Cadmium	0, 7, 11, 20, 37, 65, 114, 203	0, 7, 11, 20, 37, 65, 114, 203	0, 20, 65, 200, 650, 2032	0, 20, 65, 203, 650, 2032
Copper	0, 15, 27, 48, 150, 268, 483, 1502	0, 15, 27, 48, 150, 268, 483, 1502	0, 13, 27, 134, 268, 1342	0, 13, 27, 134, 268, 1342
Nickel	0, 23, 41, 73, 227, 405	0, 23, 41, 73, 227, 405, 728, 2266	0, 41, 130, 405, 1296, 4050	0, 41, 130, 405, 1296, 4050

2.4.3 Chemical analysis

For the 96-hour water-only tests, samples were analysed from day 0, prior to the introduction of the organisms. A sample from each concentration was poured into 20-mL scintillation vials and preserved with 2% nitric acid. Metal determination was by ICP-OES analysis (JY74 Optical Emission System) (McLaren 1981). Water samples that fell below the method detection limit for the ICP were analyzed by atomic adsorption spectrophotometry (Varian SpectraAA-400) with Zeeman background correction.

For the spiked sediment exposures, the bulk sediment, overlying water, and pore water was sampled from each chemistry beaker from day 0. Overlying water was decanted from the beakers and added to labelled scintillation vials. The bulk sediment was added to 250-mL Nalgene centrifuge tubes and centrifuged at 3750 rpm at 4°C for one hour to remove pore water. Pore water was decanted and added to labelled scintillation vials. Overlying water and pore water samples were preserved with 2% nitric acid. The bulk sediment was prepared for analysis following the procedures of Agemian and Chau (1977). Sediment samples were freeze dried, ground and homogenized, and metal determined by either ICP-OES or by AA spectrophotometry.

2.4.4 Statistical analysis

LC50's and LC25's were computed for the spiked sediment tests and the water-only tests using the trimmed Spearman-Karber method (Hamilton *et al.* 1977). The inhibition concentration estimate was performed at the 25% level on growth and reproductive endpoints of the spiked sediment tests using the linear interpolation method with confidence intervals determined using the bootstrap method (random resampling the test data with replacement) (Norberg-King 1993).

2.5 Field-Collected Sediments

2.5.1 Study areas

Three sampling areas were chosen that represented areas contaminated respectively with cadmium, nickel or copper. Sediments were collected in July/August of 1997 with a mini-ponar sampler. Five field replicate samples were collected from each site, placed in a plastic bag and stored on ice until return to the laboratory. Sites included:

1. Rouyn-Noranda Area, Québec

Five lakes were chosen to represent a range in sediment cadmium concentration from 0.2 to 15.2 μg/g. Lakes included: Opasatica (Op) (48°04'N, 79°17'W), Vaudray (Va) (48°04'N, 78°41'W), Joannès (Jo) (48°11'N, 78°41'W), D'Alembert (Dl) (48°24'N, 79°00'W) and Default (De) (48°17'N, 79°01'W).

2. Sudbury Area, Ontario

Four sites were chosen from the Onaping River and Moose Creek and were exposed to nickel mining. Nickel concentrations in sediments ranged from 24 to 342 μg/g.

Site 1 (S1)(46-43-01 N, 81-24-01 W): Depositional reference site located just below the junction of Wanitanga Creek and the Onaping River.

Site 2 (S2)(46-37-38 N, 81-23-03 W): Depositional zone located on the Onaping River just below a treated tailings input.

Site 3 (S3)(46-38-26 N, 81-23-55 W): Downstream of the Levack Sewage Treatment Plant, and above the treated tailings discharge on the Onaping River.

Site 4 (S4)(46-39-37 N, 81-21-29 W): Isolated area on Moose Creek used to store site runoff.

3. Collingwood Harbour, Ontario

Three sites (C6, C7, and C8) were collected from the East Slip of the Harbour and three sites (C9, C10, and C11) were collected from the West Slip (Figure 2.1). Copper concentrations in the sediments ranged from 59 to 525 μg/g.

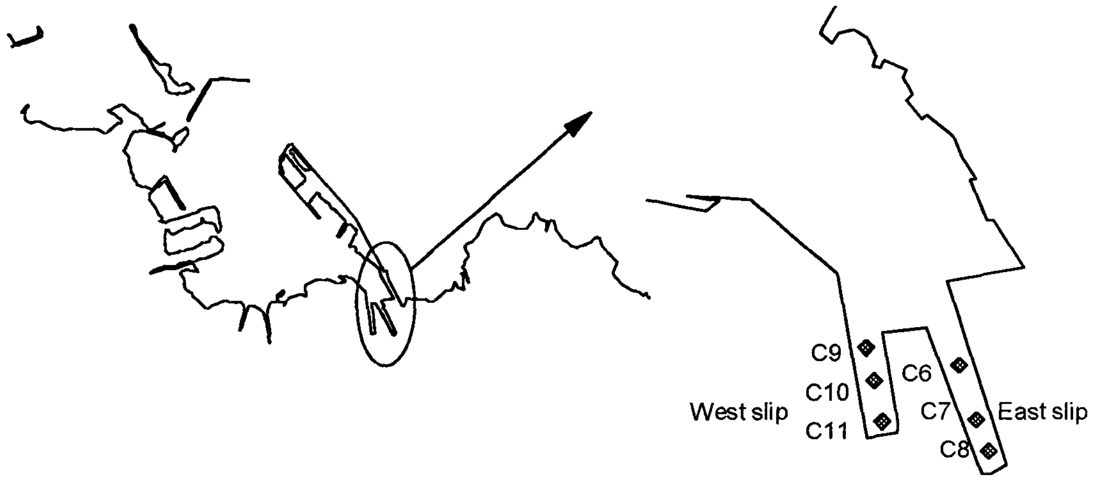

Figure 2.1 Sampling locations in Collingwood Harbour.

2.5.2 Field sediment preparation

Upon arrival to the laboratory, sediments were stored at 4°C in the dark until used for testing purposes. Prior to use, sediments were sieved to remove indigenous organisms. This is a necessary step as the presence of other organisms may affect test endpoints (Reynoldson *et al.* 1994). Each sediment sample was homogenized and wet sieved through a 250-μm sieve into plastic bags. A 4:1 ratio of culture water: sediment (2 L culture water: 500 mL sediment) was used in the sieving process. Culture water was added gradually to the sediment to produce a slurry. This slurry was then gradually poured through the 250-μm sieve and the residue discarded. The sieved sediment was allowed to settle for a minimum of 24 hours, after which the water was decanted and used as the overlying water in the tests. For sediment that did not pass through the 250-μm sieve (sandy, coarse sediment), culture water was added to the sediment and the sediment was stirred in an attempt to dislodge indigenous species into the water column. The water was then passed through the 250-μm sieve and remnants discarded.

2.5.3 Chemical analysis of field-collected sediment

Bulk sediment, overlying water, and pore water was sampled from each chemistry beaker on day 0 following procedures described in section 2.4.3.

2.5.4 Statistical analysis

Species test responses in the field-collected sediment were compared to acceptability criteria established for these four species for the Great Lakes area over a three-year period (Reynoldson *et al.* 1995,1997). Three categories (non-toxic, potentially toxic, and toxic) were developed for each endpoint based on 170 - 220 'clean' reference sediments. The non-toxic category is defined as within two standard deviations from the mean for each endpoint response. The potentially toxic category is defined as within two and three standard deviations from the mean, and the toxic category is defined as greater than three standard deviations from the mean. The comparison of species responses in sediments outside of this reference area (i.e. Rouyn-Noranda area and the Sudbury area) may not be appropriate. However, since the measured physical characteristics of these sediments fall inside the normal variation ascribed to the reference sites (Reynoldson and Day 1998b), it was deemed appropriate to make the comparison.

Multivariate statistics were performed on the field-collected data as it allows the relationship between the species test responses and the numerous environmental variables to be examined simultaneously. Multivariate techniques are an objective way to summarize data making the data easier to understand and easier to convey to others (Gauch,Jr 1989). Cluster analysis was used to identify groups (or clusters) of similar sites in the raw data, and ordination was used to reduce the number of variables (dimensions) in the data. Dissimilarity between the species responses was quantified using the Canberra metric association measure. Cluster analysis was performed using the agglomerative hierarchical fusion method with unweighted pair group mean averages. The number of groups of sites was identified by examining the dendrogram, and by examining the spatial location of the sites in ordination space. Ordination was performed by hybrid multidimensional scaling (HMDS). HMDS is a non-parametric technique that combines both metric and non metric (ranked order similarities) methods. HMDS does not have the restrictive assumptions (linearity) of other ordination techniques (Clarke and Ainsworth 1993; Clarke 1999), and is shown to be flexible and a more reliable and robust method than strictly non-metric MDS (Faith and Norris 1989; Reynoldson *et al.* 1995). The amount of scatter around the line of best fit through the HMDS distances and the dissimilarities is termed the stress coefficient (Clarke 1993). An ordination plot with a stress value of < 0.2 is considered acceptable as stress values > 0.2 are likely to be easily misinterpreted (Clarke 1993).

A multiple linear regression technique (Principal axis correlation) was used to determine how well each original attribute (test endpoints) and a second set of variables (environmental) fitted the toxicity ordination space. The significance of the relationships was tested using Monte-Carlo permutation tests. The PATN statistical package (Belbin 1993) was used for all analyses. Plots were made using Freelance Graphics (Lotus Dev. Corp. 1997). Probability ellipses were drawn around the data points using Systat (SPSS Inc. 1998).

The relationships between the test endpoints were further analysed using Spearman rank order correlation. Multiple regression (stepwise) was performed to establish the relationship between the environmental variables and each test endpoint using the software package SigmaStat (Jandel Scientific).

3. RESULTS

3.1 Water-Only/Sediment Exposures

3.1.1 96-hour water-only exposures

Table 3.1 shows the mean LC50's for cadmium, nickel, and copper for the four species in the 96-hour water-only tests. The range for each LC50 is also shown.

Table 3.1 LC50's (geometric means) (mg/L) and ranges () in 96-hour water-only exposures.

Metal	***H. azteca***	***C. riparius***	***Hexagenia spp.***	***T. tubifex***
Cadmium	0.013 (0.010-0.020)	0.021 (0.010-0.030)	7.82 (5.84-13.66)	0.87 (0.75-1.00)
Nickel	3.62 (2.10-6.19)	5.25 (2.57-8.11)	75.96[†] (73.48-80.24)	17.64 (16.27-19.86)
Copper	0.21 (0.20-0.24)	0.043 (0.040-0.050)	0.073 (0.070-0.080)	0.16 (0.12-0.24)

†

[†] May not be accurate due to the reduction in water hardness in the upper end concentrations

Cadmium

LC50's for cadmium range from 0.013 to 7.82 mg/L, a 602-fold difference between the species, which is the greatest range for the three tested metals. Of the three metals, cadmium is the most toxic (lowest LC50's) to two of the four species (*C. riparius* and *H. azteca*). The order in increasing LC50 is *H. azteca* ≤ *C. riparius* < *T. tubifex* < *Hexagenia* spp.

Nickel

Of the three metals, nickel is the least toxic metal (highest LC50's) to all four species, with LC50's ranging from 3.62 to 75.96 mg/L. As a result of the dilution of culture water with the addition of stock (made with milli-Q water), water hardness was considerable lower in the higher concentrations than in the lower concentrations in the nickel series for *Hexagenia*, thus 75.96 mg/L may not be a reliable estimate. The range in LC50's across the species is lower than cadmium (21-fold difference). Both *H. azteca* and *C. riparius* are the most sensitive species to nickel as their LC50 ranges overlap. The order in increasing LC50 is *H. azteca* = *C. riparius* < *T. tubifex* < *Hexagenia* spp.

Copper

LC50's for copper range from 0.043 to 0.16 mg/L, a 4-fold range in sensitivity between the species to this essential metal. *C. riparius* is the most sensitive species to copper, with the lowest LC50. Of the three metals, copper is most toxic (lowest LC50's) to two of the four species (*Hexagenia* spp. and *T. tubifex*). The order in increasing LC50 is *C. riparius* < *Hexagenia* spp. <

T. tubifex ≤ *H. azteca*.

Amongst species, the largest difference in sensitivity between the three metals is observed for *Hexagenia* spp. (1041-fold range), followed by *H. azteca* (278-fold difference), *C. riparius* (250-fold range), and *T. tubifex* (110-fold range).

3.1.2 Spiked-sediment exposures

Survival

The chronic LC25's and LC50's for the bulk sediment, overlying water, and pore water fractions are shown in Tables 3.2, 3.3, & 3.4 respectively.

Cadmium

Bulk sediment LC50's for cadmium range from 33 to 815 μg/g (25-fold range). Overlying water LC50's range from 0.003 to 3.56 mg/L, and pore water LC50's range from 0.013 to 3.93 mg/L. Of the three metals, cadmium is the most toxic to both *H. azteca* and *C. riparius* (LC50 ranges overlap). The order in species sensitivity is similar to what was found in the water-only tests, with *Hexagenia* and *Tubifex* now equally sensitive to cadmium (overlapping ranges). In increasing LC25/50, the order is *H. azteca* ≤ *C. riparius* << *Hexagenia spp.* = *T. tubifex*.

Nickel

Bulk sediment LC50's range from 67 to 1136 μg/g (17-fold range). Overlying water LC50's range from 0.12 to 93.79 mg/L, and pore water LC50's range from 0.27 to 111.5 mg/L. *H. azteca* has the lowest LC25/50 to nickel. The order in increasing LC25/50 is *H. azteca* < *Hexagenia* spp. < *C. riparius* < *T. tubifex*. This differs from the order in the water-only exposures. Namely, *Hexagenia* is more sensitive (lower LC25/LC50) than *Chironomus* and *Tubifex* in the sediment exposures while *Hexagenia* is the least sensitive organism (highest LC50) in water-only exposures (Table 3.1).

Copper

LC50's for copper range from 93 to 524 μg/g (6-fold range). Overlying water LC50's range from 0.021 to 0.078 mg/L, and pore water LC50's range from 0.056 to 0.668 mg/L. *Hexagenia* spp. has the lowest LC25/50 species for copper, and of the three metals copper is most toxic to *Hexagenia* spp. and *T. tubifex*. The order in increasing LC25/50 is *Hexagenia* spp. = *H. azteca* < *C. riparius* < *T. tubifex*. This differs from the order in the water-only exposures. Namely, *Hyalella* is the least sensitive organism (highest LC50) in the water-only exposures (Table 3.1) while in the sediment exposures *Hyalella* is one of the most sensitive organisms (≡ *Hexagenia*).

Amongst species, the largest difference (in the bulk sediment fraction) in sensitivity between the three metals exists for *C. riparius* (17-fold), followed by *Hexagenia spp.* (9-fold), *H. azteca* (4-fold), and *T. tubifex* (2-fold) (Table 3.2).

Table 3.2 Chronic LC25's and LC50's (geometric means) (µg/g) and ranges () based on bulk sediment metal concentrations.

Time		*28d*	*10d*	*21d*	*28d*
Metal		***H. azteca***	***C. riparius***	***Hexagenia spp.***	***T. tubifex***
Cadmium	LC25	21 (16 - 32)	28 (26 - 30)	560 (357 - 752)	600 (526 – 702)
	LC50	33 (28 - 44)	39 (36 - 46)	815 (595 - 1024)	787 (715 - 931)
Nickel	LC25	48 (43 – 57)	505 (437 - 578)	324 (275 – 377)	918 (807 – 1107)
	LC50	67 (62 - 74)	665 (593 – 753)	452 (373 - 559)	1136 (1006 - 1380)
Copper	LC25	81 (57 – 106)	265 (191 - 318)	60 (55 – 65)	349 (300 – 393)
	LC50	128 (110 - 158)	402 (307 - 488)	93 (90 - 98)	524 (478 - 567)

Table 3.3 Chronic LC25's and LC50's (geometric means) (mg/L) and ranges () based on overlying water metal concentrations.

Time		*28d*	*10d*	*21d*	*28d*
Metal		***H. azteca***	***C. riparius***	***Hexagenia spp.***	***T. tubifex***
Cadmium	LC25	0.0023 (0.0022 - 0.0023)	0.0025 (0.0019 - 0.0030)	0.48 (0.19 - 1.19)	1.14 (0.35 - 5.84)
	LC50	0.0032 (0.0029 - 0.0035)	0.0033 (0.0027 - 0.0045)	3.09 (0.87 - 14.28)	3.56 (1.03 - 14.86)
Nickel	LC25	0.071 (0.060 - 0.11)	2.85 (2.76 - 2.92)	2.10 (1.06 - 3.34)	42.10 (21.92 - 73.27)
	LC50	0.12 (0.082 - 0.18)	9.89 (9.53 - 10.27)	5.07 (2.71 - 8.49)	93.79 (61.75 - 142.00)
Copper	LC25	0.012 (0.0091 - 0.020)	0.041 (0.035 - 0.053)	0.018 (0.0074 - 0.030)	0.057 (0.048 - 0.073)
	LC50	0.021 (0.012 - 0.038)	0.070 (0.046 - 0.092)	0.027 (0.018 - 0.036)	0.078 (0.061 - 0.103)

Table 3.4 Chronic LC25's and LC50's (geometric means) (mg/L) and ranges () based on pore water metal concentrations.

Time		*28d*	*10d*	*21d*	*28d*
Metal		***H. azteca***	***C. riparius***	***Hexagenia spp.***	***T. tubifex***
Cadmium	LC25	0.0069 (0.0046 - 0.015)	0.012 (0.0067 - 0.022)	0.79 (0.33 - 2.86)	1.15 (0.75 - 1.69)
	LC50	0.013 (0.0070 - 0.026)	0.018 (0.012 - 0.026)	3.93 (1.23 - 20.99)	3.73 (2.63 - 6.68)
Nickel	LC25	0.17 (0.10 - 0.35)	7.38 (6.22 - 8.39)	4.02 (2.80 - 5.66)	56.36 (45.69 - 74.81)
	LC50	0.27 (0.14 - 0.56)	17.94 (15.92 - 19.54)	8.86 (7.13 - 11.88)	111.50 (96.48 - 143.80)
Copper	LC25	0.034 (0.031 - 0.038)	0.113 (0.074 - 0.151)	0.034 (0.031 - 0.036)	0.487 (0.241 - 0.770)
	LC50	0.056 (0.053 - 0.057)	0.182 (0.118 - 0.227)	0.060 (0.057 - 0.063)	0.668 (0.321 - 1.033)

The chronic pore water and overlying water LC50's are lower than the acute LC50's for all metals for *Hyalella* and *Hexagenia* (with the exception of the pore water cadmium LC50 for *Hyalella* which is the same as the acute value) (Tables 3.1, 3.3, & 3.4). For *Chironomus*, the chronic pore water and overlying water LC50's are higher than the acute LC50's for both nickel and copper (with the exception of the cadmium pore water and overlying water LC50's that are similar or lower than the acute LC50). For *Tubifex*, the chronic LC50's are higher than the acute LC50's for all metals, with the exception of the copper overlying water LC50 (Table 3.3), which is slightly lower than the acute LC50 (Table 3.1).

Growth and Reproduction

IC25's for the bulk sediment, overlying water and pore water fractions are shown in Tables 3.5, 3.6 & 3.7 respectively. An overall impairment in growth and reproduction occurs with increasing metal concentrations.

Cadmium

In the bulk sediment fraction, there is a narrow range in IC25's for *Hyalella*, *Chironomus* and *Hexagenia* growth (10 to 16 μg/g). IC25's for *Tubifex* reproduction endpoints are higher than the growth endpoints, ranging from 301 to 769 μg/g. Overlying water IC25's for growth range from 0.001 to 0.003 mg/L, and from 0.21 to 17.1 mg/L for reproduction endpoints. Pore water IC25's for growth range from 0.003 to 0.010 mg/L, and from 0.28 to 17.4 mg/L for reproduction endpoints. Young production is the most sensitive reproduction endpoint for *Tubifex*, as seen by the lowest IC25. Of the three metals, cadmium is most toxic to *Hyalella*, *Chironomus* and *Hexagenia*. The order in increasing IC25's is *H. azteca* ≤ *Hexagenia* spp. ≤ *C. riparius* << *T. tubifex*. This differs from the order observed for survival, where the LC25's for *Hexagenia* are 27 to 209-fold higher than the LC25's for *Hyalella* (Tables 3.2, 3.3 & 3.4).

Nickel

Based on the bulk sediment fraction the IC25's for growth range from 40 to 146 µg/g, overlying water IC25's range from 0.03 to 0.34 mg/L, and pore water IC25's range from 0.11 to 1.16 mg/L. Of the three metals, nickel IC25's are highest for *Hexagenia* and *Chironomus* growth. IC25's for reproduction are higher than for growth, ranging from 408 to 669 µg/g in the bulk sediment, from 6.5 to 26.0 mg/L in the overlying water, and from 10.2 to 31.9 mg/L in the pore water. Nickel is the least toxic metal to *Tubifex* young reproduction of the three metals (highest IC25's), while the IC25's for cocoon production and the percent of cocoons hatched are close to the IC25's for cadmium. The order in increasing IC25's is *H. azteca* < *Hexagenia* spp. < *C. riparius* < *T. tubifex*, which is the same order observed for survival LC25's (Table 3.2).

Copper

For growth, bulk sediment IC25's range from 38 to 78 µg/g, overlying water IC25's range from 0.012 to 0.024 mg/L, and pore water IC25's range from

0.020 to 0.047 mg/L. IC25's for reproduction are higher than for growth, ranging from 181 to 266 μg/g in the bulk sediment, from 0.037 to 0.053 mg/L in the overlying water, and from 0.22 to 0.36 mg/L in the pore water. Of the three metals, copper is most toxic to *Tubifex* reproduction (lowest IC25's). The IC25 for young production and percent cocoons hatched are similar, while the IC25 for cocoon production is slightly higher. The order in increasing sensitivity to copper is *Hexagenia* spp. ≤ *H. azteca* ≤ *C. riparius* < *T. tubifex*, which is the same order observed for survival LC25's (Table 3.2).

Table 3.5 IC25's (geometric means) (μg/g) and ranges () for growth and reproduction endpoints based on bulk sediment metal concentrations.

	Growth			**Reproduction (*T. tubifex*)**		
Metal	***H. azteca***	***C. riparius***	***Hexagenia* spp.**	**Number Young per Adult**	**Number Cocoons Per Adult**	**Percent Cocoons Hatched**
Cadmium	10 (6 – 18)	16 (14 - 20)	14 (8 – 25)	301 (259 – 336)	467 (330 – 688)	769 (690 – 938)
Nickel	40 (31 – 57)	146 (97 - 204)	83 (82 – 86)	408 (347 – 514)	451 (369 – 618)	669 (430 – 911)
Copper	76 (72 - 78)	78 (35 – 143)	38 (31 - 49)	181 (161 - 220)	266 (185 - 493)	185 (163 - 231)

Table 3.6 IC25's (geometric means) (mg/L) and ranges () for growth and reproduction endpoints based on overlying water metal concentrations.

	Growth			**Reproduction (*T. tubifex*)**		
Metal	***H. azteca***	***C. riparius***	***Hexagenia* spp.**	**Number Young per Adult**	**Number Cocoons Per Adult**	**Percent Cocoons Hatched**
Cadmium	0.0010 (0.0010 - 0.0011)	0.0014 (0.0010 - 0.0022)	0.0032 (0.0010 - 0.014)	0.21 (0.07 - 0.53)	1.09 (0.11 - 5.98)	17.10 (6.64 - 28.11)
Nickel	0.034 (0.015 - 0.054)	0.34 (0.16 – 0.64)	0.13 (0.079 - 0.22)	6.47 (3.16 - 11.16)	7.97 (4.45 - 12.98)	26.01 (15.23 - 68.95)
Copper	0.012 (0.0083 - 0.014)	0.024 (0.020 - 0.030)	0.017 (0.012 - 0.029)	0.037 (0.026 - 0.051)	0.053 (0.040 - 0.065)	0.038 (0.028 - 0.051)

Table 3.7 IC25's (geometric means) (mg/L) and ranges () for growth and reproduction endpoints based on pore water metal concentrations.

	Growth			**Reproduction (*T. tubifex*)**		
Metal	***H. azteca***	***C. riparius***	***Hexagenia spp.***	**Number Young per Adult**	**Number Cocoons Per Adult**	**Percent Cocoons Hatched**
Cadmium	0.0030 (0.0018 - 0.0076)	0.0056 (0.0035 - 0.0082)	0.0096 (0.0047 - 0.016)	0.28 (0.24 - 0.35)	1.21 (0.57 - 5.40)	17.39 (6.44 - 30.57)
Nickel	0.11 (0.031 - 0.37)	1.16 (0.46 - 2.11)	0.65 (0.42 - 0.89)	10.21 (8.46 - 12.59)	12.48 (11.32 - 14.39)	31.87 (16.62 - 66.32)
Copper	0.030 (0.024 - 0.037)	0.047 (0.045 - 0.050)	0.020 (0.012 - 0.026)	0.22 (0.11 - 0.38)	0.36 (0.13 - 0.89)	0.22 (0.11 - 0.42)

3.2 Field-Collected Sediments

The physical characteristics and metal concentrations for field-collected sediments are shown in Table 3.8. The Ontario and the Canadian sediment assessment values for the measured metals are included in Table 3.8 for reference. Mean species survival, growth, and reproduction in field-collected sediments is shown in Table 3.9. The established criteria for each category (non-toxic, potentially toxic and toxic) for each species are also included in Table 3.9. Toxicity is highlighted in bolded text, and potential toxicity is highlighted in italicised text.

Table 3.8 Physical and chemical characteristics of field sediments. Threshold and probable effect levels (TEL/PEL) and lowest and severe effect level (LEL/SEL) are included for reference.

Location	Site ()[][1]	TOC (%)	% Silt	% Clay	% Sand	Al %	Cd µg/g	Cr µg/g	Cu µg/g	Fe %	Mg %	Mn %	Ni µg/g	Pb µg/g	Zn µg/g	pH range
Collingwood Harbour	C6 (2)[3]	1.9	71.8	26.1	2.1	7.2	<1.0	30	357	1.6	5.2	0.07	10	123	1120	7.5-8.6
	C7 (2)[3]	2.0	73.3	25.5	1.2	8.2	<1.0	34	335	1.6	3.0	0.08	12	134	1119	7.6-8.4
	C8 (2)[4]	2.2	69.8	28.5	1.8	7.6	<1.0	103	525	2.3	2.7	0.09	9	205	1625	7.6-8.5
	C9 (0)[1]	1.7	73.2	21.5	5.3	8.2	<1.0	26	59	0.8	3.4	0.08	7	189	301	7.7-9.5
	C10 (0)[1]	1.8	73.2	23.7	3.1	7.6	<1.0	29	69	0.8	3.4	0.08	8	166	231	7.7-8.6
	C11 (0)[1]	1.7	64.4	19.9	15.6	8.1	<1.0	31	86	0.8	3.6	0.08	8	252	292	7.7-8.5
Sudbury	S 1 (0)[0]	0.1	2.8	2.8	94.4	4.8	<3.4	45	14	1.7	0.6	0.04	24	<2.5	58	6.4-7.8
	S 2 (1)[1]	0.2	2.4	2.5	95.3	5.0	<3.4	48	91	1.6	0.6	0.03	144	<2.5	51	5.1-7.5
	S 3 (0)[0]	0.1	8.3	8.3	81.2	5.2	<3.4	45	8	1.6	0.7	0.03	27	<2.5	45	5.3-7.5
	S 4 (2)[4]	0.2	28.6	5.1	64.5	5.0	9.9	103	799	10.9	2.3	0.07	342	2.8	95	2.8-4.6
Rouyn-Noranda	OP (1)[2]	0.1	81.0	14.9	4.1	6.4	0.2	118	36	3.5	2.3	0.08	61	<2.5	101	5.7-8.5
	VA (0)[0]	0.1	37.5	50.4	12.1	5.3	<3.4	68	13	2.0	0.8	0.09	28	<2.5	77	6.0-8.3
	JO (0)[0]	0.1	41.0	25.4	33.6	4.6	<3.4	49	12	1.3	0.5	0.05	17	<2.5	62	5.9-7.8
	DL (0)[0]	0.1	47.5	15.0	37.5	5.5	<3.4	63	56	2.2	0.9	0.05	29	<2.5	81	5.5-7.8
	DE (4)[5]	0.2	72.8	22.9	4.3	5.6	15.2	77	1317	3.4	1.0	0.05	38	267	1728	4.8-7.6
	TEL[2]	-	-	-	-	-	0.6	37.3	35.7	-	-	-	18	35	123	-
	PEL[2]	-	-	-	-	-	3.5	90	197	-	-	-	36	91.3	315	-
	LEL[3]	-	-	-	-	-	0.6	26	16	-	-	-	16	31	120	-
	SEL[3]	-	-	-	-	-	10	110	110	-	-	-	75	250	820	-

[1] number of criteria exceeded () = provincial (SEL), [] = federal (PEL); [2] from Smith *et al.* (1996); [3] from Persaud *et al.* (1992)

Table 3.9 Mean survival, growth, and reproduction and standard deviations () in field sediments and in spiked-sediment data points Cd3, Ni3, and Cu3. Criteria for determining toxicity for nearshore sediments of the Great Lakes (from Reynoldson and Day 1998) are included for reference.

	H. azteca		***C. riparius***		***Hexagenia spp.***		***T. tubifex***			
SITE	**Survival**	**Growth**	**Survival**	**Growth**	**Survival**	**Growth**	**Survival**	**Number of Cocoons/Ad**	**Percent Hatched**	**Number of Young/Ad**
C6	80.0 (19.4)	0.33 (0.16)	95.0 (3.3)	*0.19 (0.04)*	100.0 (0)	6.28 (0.79)	95.0 (11.2)	10.2 (0.7)	**14.9 (7.8)**	*2.2 (2.2)*
C7	77.3 (22.4)	0.31 (0.09)	85.3 (8.7)	0.21 (0.03)	98.0 (4.5)	4.77 (1.55)	100.0 (0)	9.8 (1.6)	**16.0 (5.4)**	**0.10 (0.1)**
C8	*66.7 (27.2)*	0.23 (0.21)	*61.7 (33.3)*	*0.19 (0.09)*	100.0 (0)	5.80 (2.27)	100.0 (0)	10.8 (0.6)	*29.2 (18.9)*	11.2 (4.9)
C9	78.3 (16.7)	0.24 (0.07)	70.0 (12.8)	0.28 (0.09)	96.0 (8.9)	2.76 (1.80)	100.0 (0)	9.2 (0.6)	**22.0 (9.4)**	*4.3 (5.6)*
C10	95.0 (6.4)	0.21 (0.09)	85.3 (7.3)	0.32 (0.02)	98.0 (4.5)	4.37 (1.56)	100.0 (0)	9.8 (0.9)	**16.0 (12.9)**	*3.4 (2.9)*
C11	92.0 (8.7)	0.30 (0.15)	90.7 (11.2)	0.28 (0.04)	100.0 (0)	3.38 (1.75)	95.0 (11.2)	8.8 (1.4)	38.5 (10.5)	10.3 (3.5)
S1	**22.7 (31.8)**	**0.03 (0.01)**	88.3 (11.4)	0.41 (0.08)	96.0 (5.5)	3.46 (0.89)	100.0 (0)	10.8 (1.0)	57.7 (3.2)	38.1 (4.6)
S2	**8.0 (17.9)**	**0.02 ()**	80.0 (17.0)	0.36 (0.13)	*84.0 (18.2)*	*0.29 (0.47)*	100.0 (0)	10.8 (0)	52.3 (1.6)	35.8 (1.8)
S3	**51.7 (23.9)**	*0.12 (0.11)*	89.3 (7.6)	0.44 (0.06)	96.0 (5.5)	1.97 (0.58)	100.0 (0)*	11.3 ()*	53.3 ()*	43.3 ()*
S4	**0 (0)**	-	**48.0 (12.8)**	**0.13 (0.05)**	**8.0 (8.4)**	**-0.67(0.26)**	**15.0 (33.5)**	**0 (0)**	-	**0.6 (1.3)**
OP	**25.3 (21.8)**	0.32 (0.16)	74.7 (15.9)	0.40 (0.10)	94.0 (13.4)	2.12 (0.12)	95.0 (11.2)	8.1 (1.4)	69.6 (10.2)	13.0 (5.9)
VA	**40.0 (25.4)**	*0.14 (0.01)*	**54.7 (7.3)**	0.39 (0.17)	100.0 (0)	3.88 (0.33)	100.0 (0)	9.8 (0.5)	54.0 (3.2)	15.8 (4.7)
JO	**32.0 (32.5)**	0.24 (0.11)	**58.7 (33.5)**	0.41 (0.12)	100.0 (0)	3.08 (0.31)	100.0 (0)	9.1 (1.0)	54.8 (9.5)	20.4 (5.9)
DL	86.7 (7.7)	*0.19 (0.04)*	85.3 (3.0)	0.31 (0.13)	96.0 (8.9)	3.35 (0.46)	100.0 (0)	10.0 (1.2)	58.1 (4.6)	20.7 (4.4)
DE	**0 (0)**	-	76.0 (21.9)	**0.11 (0.03)**	**78.0 (16.4)**	**-0.35(0.19)**	**80.0 (20.9)**	*6.7 (1.3)*	100.0 (0)	*5.9 (4.5)*
Cd3	**0 (0)**	-	**0 (0)**	-	96.9 (3.4)	*0.13 (0.12)*	97.9 (3.6)	9.5 (0.8)	63.0 (1.2)	23.1 (2.3)
Ni3	**0 (0)**	-	93.3 (3.4)	*0.20 (0.03)*	90.6 (4.2)	*0.72 (0.48)*	100 (0)	10.3 (1.1)	55.0 (5.1)	28.8 (12.2)
Cu3	**0 (0)**	-	*65.6 (21.7)*	**0.11 (0.01)**	**5.0 (0)**	**-0.59 (0.17)**	95.8 (7.2)	*6.8 (2.5)*	**25.9 (15.8)**	*7.2 (5.1)*
NT	≥ 67.0	0.75 – 0.23	≥ 67.7	0.49 - 0.21	≥ 85.5	5.04 - 0.97	> 88.9	12.4 - 7.2	78.1 - 38.1	46.3 - 9.9
PT	67.0 - 57.1	0.22 – 0.10	67.7 - 58.8	0.20 - 0.14	85.4 - 80.3	0.96 - 0.0	88.9 - 84.2	7.1 - 5.9	38.0 - 28.1	9.8 - 0.8
T	< 57.1	< 0.10	< 58.8	< 0.14	< 80.3	-	< 84.2	< 5.9	< 28.1	< 0.8

NT= non-toxic; PT= possible toxicity; T= toxicity

3.2.1 Collingwood Harbour

Collingwood Harbour sediments (sites C6, C7, C8, C9, C10, and C11), consist of a high percentage of silt and clay, and have a total organic carbon content ranging from 1.7 to 2.2%. C11 sediment has the highest sand content (15.6%) and lowest percent clay (19.9%) (Table 3.8). Copper concentrations in the sediments range from 59 to 525 μg/g, and notably, sediments also contain high concentrations of zinc (292 - 1625 μg/g) and lead (123 - 252 μg/g). C8 sediment has the overall highest chromium, copper, iron, and zinc concentrations (Table 3.8). Comparing the sediment metal concentrations to Environment Canada's sediment assessment values (Smith *et al.* 1996), shows C6, C7, & C8 sediments to be above the probable effects level (PEL) for zinc (315 μg/g), and copper (197 μg/g). All sediments are above the PEL for lead (91.3 μg/g) and C8 is above the PEL for chromium (90 μg/g) (Table 3.8).

Mean survival data indicate potential toxicity to *Hyalella* and *Chironomus* in C8 sediment. No toxicity or potential toxicity to survival is evident for *Hexagenia* or *Tubifex* in any sediment (Table 3.9). The sublethal responses (growth and reproduction) show C6 and C8 sediments to be potentially toxic to *Chironomus*. *Tubifex* reproduction appears to be the most affected in the Collingwood Harbour sediments, with toxicity and/or potential toxicity observed for percent hatch and young production in all sediments except C11. The sediment producing the greatest number of young/adult (C8) (Table 3.9), has the highest concentration of copper (525 μg/g), zinc (1625 μg/g), chromium (103 μg/g), and the second highest concentration of lead (205 μg/g) (Table 3.8).

3.2.2 Sudbury area

Sediments from the Sudbury area (sites S1, S2, S3, and S4) contain a high percent of sand particles, ranging from 65 to 95%. S4 sediment has the lowest percent sand, the highest percent silt (28.6%), and the highest concentrations of cadmium, chromium, copper, iron, and nickel. Organic carbon is low in all sediments, ranging from 0.1 to 0.2% (Table 3.9). Nickel concentrations in the sediments range from 24 to 342 μg/g, and copper concentrations range from 8 to 800 μg/g. Cadmium, chromium, copper, and nickel concentrations in S4 sediment are above the PEL's, and the nickel concentration in S2 is above the PEL (Table 3.8).

S4 sediment is toxic to all four species. Mean survival data show extreme toxicity to *Hyalella* (zero *Hyalella* survival) and low survival to the other three species. There is also toxicity evident to *Hyalella* survival in S1, S2, and S3 sediments, and potential toxicity to *Hexagenia* survival in S2. The sublethal responses show the same pattern of toxicity as the survival data. S4 is toxic to all species (growth and reproduction), S1, S2, and S3 sediments show toxicity to *Hyalella* growth, and S2 sediment shows potential toxicity to *Hexagenia* growth (Table 3.9). Site S4 had a low pH (3.9) on the day of sampling, and a pH ranging from 2.8 to 4.6 in the overlying water in the test beakers throughout the tests.

3.2.3 Rouyn-Noranda area

Of the Rouyn-Noranda sediments (sites Op, Va, Jo, Dl, De), Lake Default (De), has the highest cadmium concentration (15.2 μg/g), and also has a high concentration of copper (1317 μg/g), lead (267 μg/g) and zinc (1728 μg/g) (Table 3.8). Cadmium concentration in the other sediments are low (0.2 μg/g in Lake Opasatica (Op)), or < 3.4 μg/g in Lakes Vaudray (Va), Joannès (Jo), and D'Alembert (Dl). All sediments have a low organic carbon content (0.1 to 0.2%), and consist mainly of silt and clay, with the exception of Jo and Dl sediments, which consist mainly of silt and sand (Table 3.8). Cadmium, nickel, lead, and zinc concentrations in De are above the PEL's, and chromium and nickel concentrations are above the PEL's in Op (Table 3.8).

Mean survival data show that sediment from site De is toxic to *Hyalella* (zero survival), *Hexagenia*, and *Tubifex*. Toxicity is also evident to *Hyalella* in Op, Va, and Jo sediments, and to *Chironomus* in Va and Jo sediments. Mean sublethal responses show toxicity to *Hyalella*, *Chironomus*, and *Hexagenia* growth, and potential toxicity to *Tubifex* reproduction in De sediment. Potential toxicity is also evident to *Hyalella* in Va and Dl sediment.

Figure 3.1 shows the ordination of the species test responses in the field-collected sediments reduced on two axes. Data for each metal from the spiked sediment exposures are also included. These spiked data represent the species response to each metal separately thereby showing whether the species respond similarly to each metal or not. For each metal the three concentrations in the spiked exposures that were common to the four species are shown. Points Cd1, Cd2, and Cd3 represent mean species test responses in sediment with mean cadmium concentrations of 1, 19, and 158 μg/g respectively. Points Ni1, Ni2, and Ni3 represent mean species test responses in sediments with mean nickel concentrations of 5, 32, and 271 μg/g respectively, and the points Cu1, Cu2, and Cu3 represent mean species test responses in sediment with mean copper concentrations of 12, 38, and 255 μg/g respectively. Mean species responses in the spiked points consisting of the highest metal concentrations (Cd3, Ni3, and Cu3) are shown in Table 3.9.

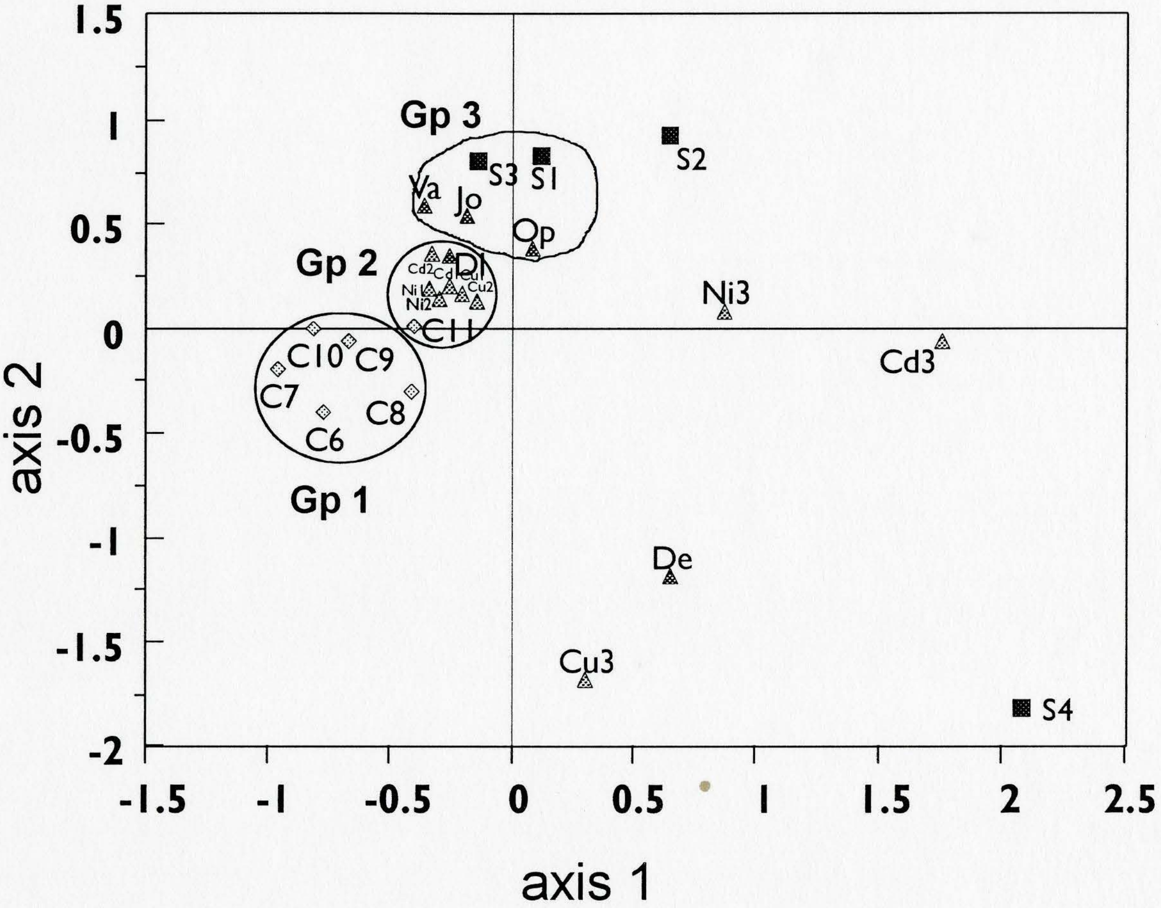

Figure 3.1 Ordination plot (MDS) of field and spiked data showing the three groups (Gp) formed by cluster analysis (stress level = 0.12). ◇= Collingwood Harbour sites, ▲= Rouyn-Noranda sites, ■= Sudbury sites, △= spiked points.

The degree of similarity among the sites in the ordination is represented by their spatial proximity. Collingwood Harbour sites are in close proximity to each other, indicating that their degree of toxicity and the type of response are similar for these sites. Rouyn-Noranda sites also lie in close proximity of each other indicating their similarity, with the exception of site De that is positioned away from the other Rouyn-Noranda sites. De is the most toxic of the Rouyn-Noranda sediments. Sudbury sites S1 and S3 are closest to each other, indicating their similarity while S2 is positioned farther away, and S4 (the most toxic of the Sudbury sediments) is quite different from the other Sudbury sediments, lying far apart from them. The spiked data points consisting of the lower metal concentrations (Cd1, Cd2, Cu1, Cu2, Ni1, and Ni2) are very similar to each other and are in close proximity to each other on the graph. The three spiked data points that contain the highest concentration of each metal (Cd3, Cu3 & Ni3) are highly variable, and differ from the rest of the spiked points. Spiked points Cd3 and Ni3 appear to behave similarly, showing variation along the first axis, while Cu3 is influenced by the second axis.

Cluster analysis identifies three possible group formations from the dendrogram (Figure 3.2).

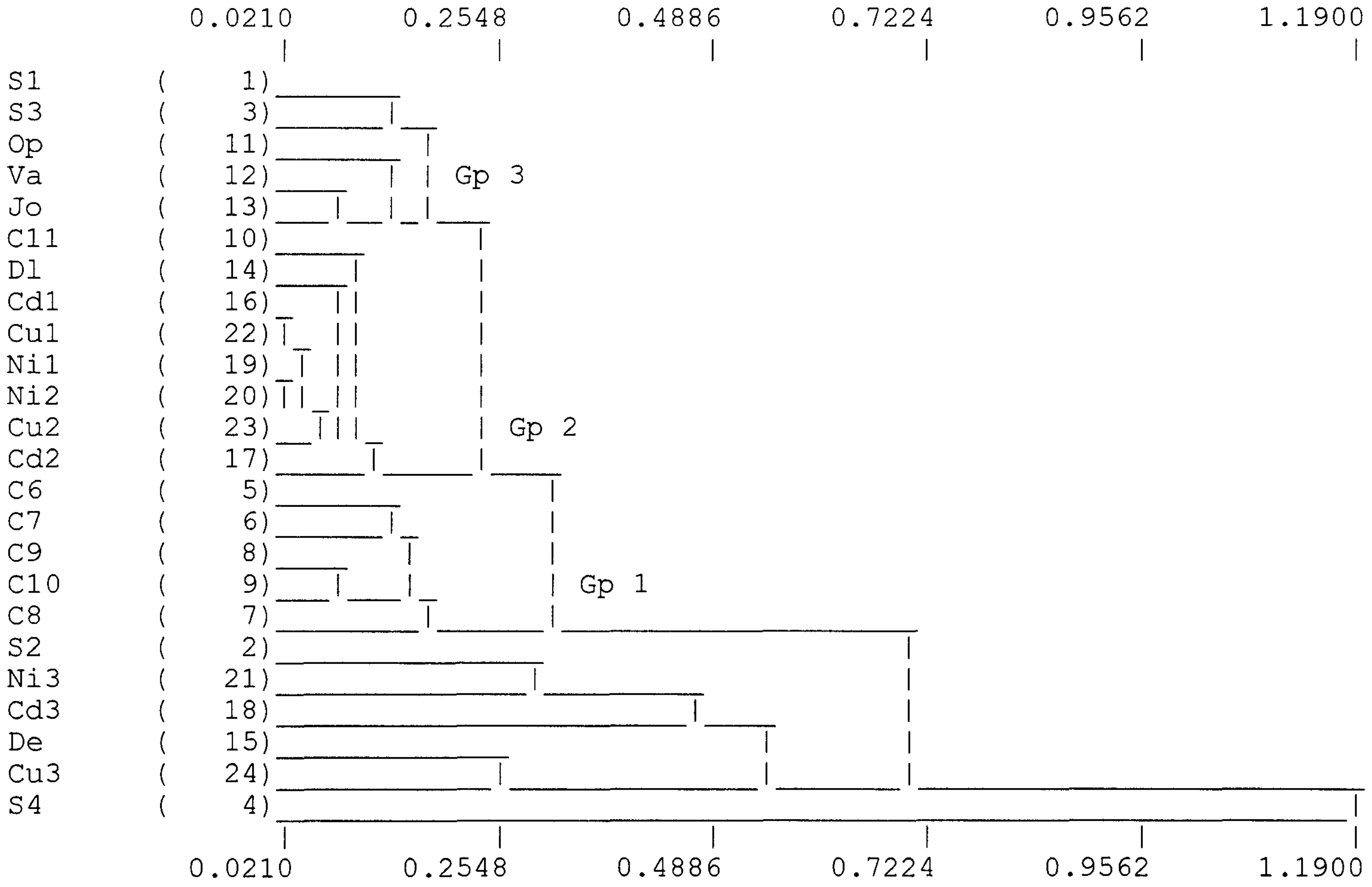

Figure 3.2 *Dendrogram of field and spiked data showing the group designations.*

The group (Gp) numbers in Figure 3.1 represent these three group formations. Each cluster represents similarities in species test responses between the sites in the cluster and the smaller the cluster, the more similar the sites. Sites forming group 1, (all Collingwood Harbour sites with the exception of C11) are toxic to *Tubifex* (impaired reproduction) (Table 3.9), and contain elevated zinc, copper, and lead concentrations (Table 3.8). Sites forming group 2, (Cd1&2, Ni1&2, Cu1&2, DI, and C11) represent those which show no toxicity to any species (only potential toxicity to *Hyalella* growth in DI sediment) (Table 3.9). Group 3 (sites S1, S3, Op, Va, & Jo) are sediments that show toxicity to either *Hyalella* only or to *Hyalella* and *Chironomus* only (Table 3.9). The remaining sites (De, Cd3, Ni3, Cd3, and S4) show the most variation in test response, are overall the most toxic sediments to all four species (Table 3.9), and are the sediments that contain overall the highest measured metal concentrations (Table 3.8). Groups 1, 2 & 3 are confined to smaller areas in ordination space, and the sites within each group are all similar, whereas the remaining sites are distributed variably and are quite different from each other.

Figure 3.3 shows the relationship between test endpoints and the field sites. The arrows (or vectors) indicate the direction of maximum correlation. Table 3.10 shows the contribution of each test endpoint to the ordination from principal axis correlation.

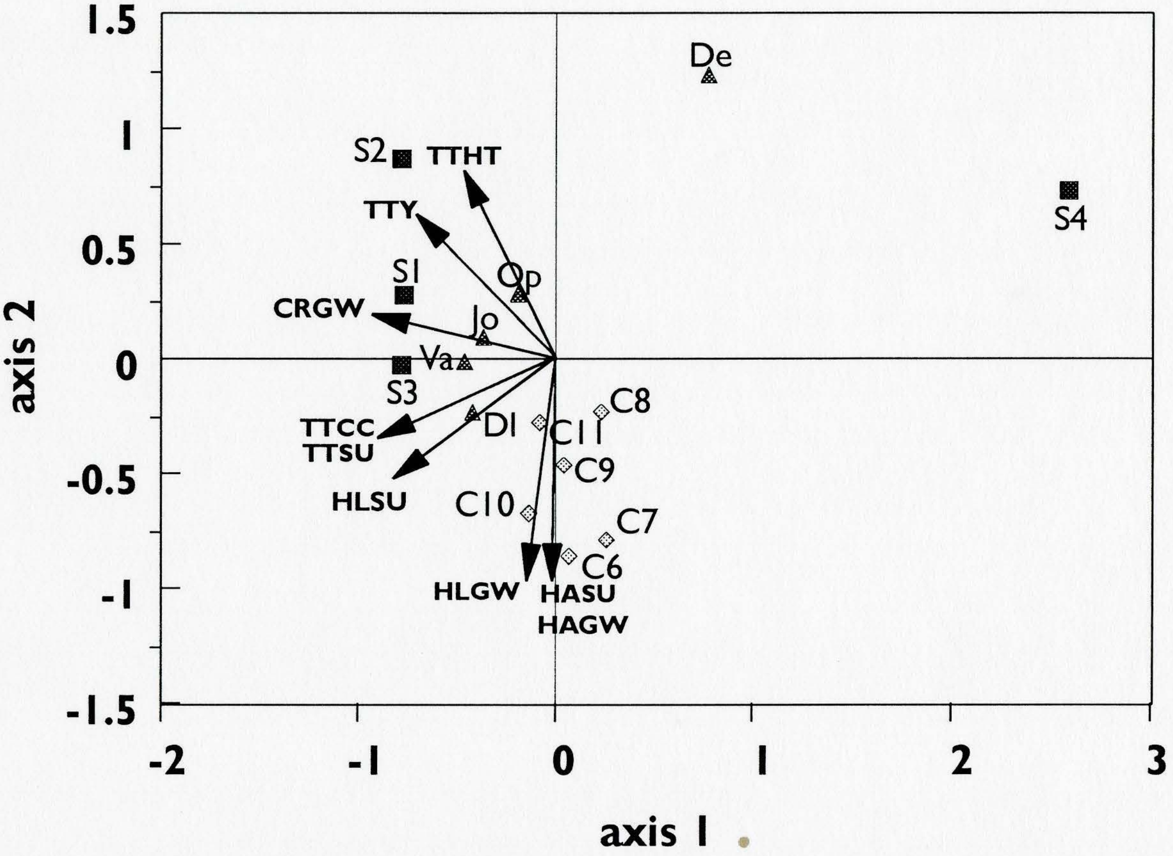

Figure 3.3 Ordination plot (MDS) of field data. Arrows indicate the direction in which the test endpoints (significant at $p \leq 0.05$) influence the ordination pattern (stress level = 0.11). TTSU=Tubifex survival, TTCC=Tubifex cocoon production, TTHT=Tubifex percent cocoon hatch, TTY=Tubifex young production, CRGW=Chironomus growth, HLSU=Hexagenia survival, HLGW=Hexagenia growth, HASU=Hyalella survival, HAGW=Hyalella growth.

Table 3.10 Contribution of test endpoints to the ordination vectors by principal axis correlation.

Endpoint	Abbreviation	r
Hyalella survival	HASU	0.9185
Hyalella growth	HAGW	0.7894
Chironomus survival	CRSU	0.5192
Chironomus growth	CRGW	0.7963
Hexagenia survival	HLSU	0.9131
Hexagenia growth	HLGW	0.8811
Tubifex survival	TTSU	0.9383
Tubifex cocoon production	TTCC	0.9464
Tubifex percent hatch	TTHT	0.8230
Tubifex young production	TTY	0.8257

All test endpoints were significant based on Monte-Carlo analysis ($p \leq 0.05$) with the exception of *Chironomus* survival. Spearman rank order correlation, performed on the test endpoints and shown in Table 3.11, reveals several relationships. There are significant relationships between *Hyalella* survival and growth (HASU/HAGW) and *Hexagenia* survival (HLSU) and *Hexagenia* growth (HLGW), and between *Hexagenia* survival and growth, which corresponds well to the location of these vectors in the lower left corner of the MDS ordination (Figure 3.3). There is also a significant relationship between *Tubifex* survival (TTSU) and cocoon production (TTCC), and between *Tubifex* cocoon hatch (TTHT) and young production (TTY), also corresponding well to the location of these vectors in Figure 3.3. The relationship between

Chironomus growth (CRGW) and *Tubifex* survival and *Tubifex* young production is also significant based on Spearman rank order correlation. The location of the *Tubifex* cocoon hatch and young production vectors (upper left corner) and the Collingwood Harbour sites (lower right corner) reveals a relationship. Depressed percent cocoon hatch and young production is evident in all Collingwood Harbour sediments with the exception of C11 (Table 3.9), while S1 and S2 sediments produced a high percent cocoon hatch and young production. Orthogonal to these vectors are the *Hexagenia* survival, *Tubifex* survival and *Tubifex* cocoon vectors (lower left corner), which show a relationship with sites De and S4 (upper right corner). Lowest mayfly and worm survival and cocoon production are evident in De and S4 sediments (Table 3.9). *Hexagenia* growth and *Hyalella* survival and growth follow the direction of the Collingwood Harbour sites, and these sediments produced the highest survival and growth for these two organisms.

Table 3.11 Spearman rank order correlation for test endpoints.

Endpoint	Significant Relationship(s)	Correlation Coefficient	p value
Hyalella survival	*Hyalella* growth	0.609	0.016
	Hexagenia survival	0.622	0.013
	Hexagenia growth	0.649	0.008
Hyalella growth	*Hexagenia* survival	0.603	0.017
	Hexagenia growth	0.594	0.019
Hexagenia survival	*Hexagenia* growth	0.814	0.000
Chironomus growth	*Tubifex* survival	0.555	0.031
	Tubifex young production	0.766	0.000
Tubifex survival	*Tubifex* cocoon production	0.674	0.005
Tubifex cocoon production	*Tubifex* young production	0.529	0.041
Tubifex percent cocoon hatch	*Tubifex* young production	0.670	0.006

Figure 3.4 shows the relationship between the field data and the environmental variables in ordination space. Missing environmental data (values that fell below the detection limits) were replaced with random values between 0 and just below the detection limit to avoid unreal weighting of a particular compound in the analysis. The arrows (or vectors) indicate the direction of maximum correlation. Table 3.12 shows the contribution of the environmental variables to the ordination from principal axis correlation.

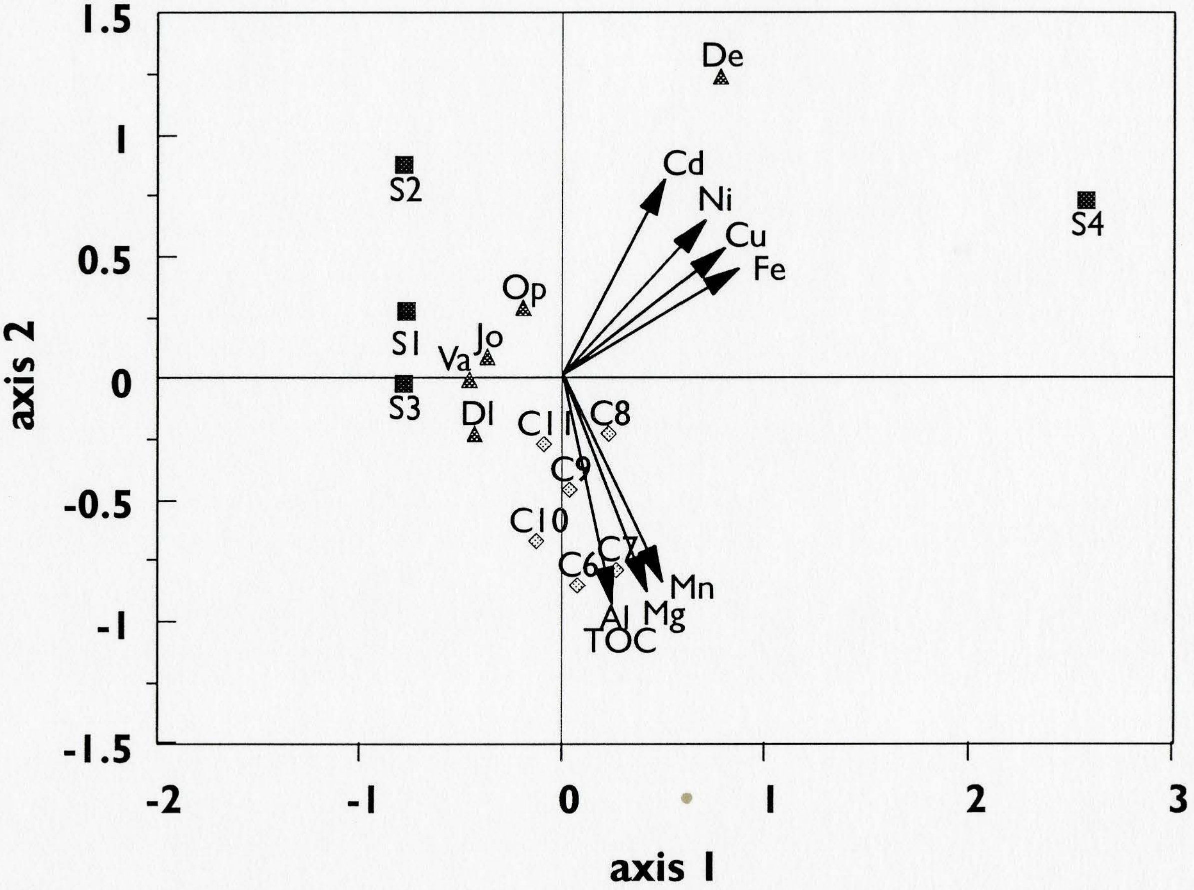

Figure 3.4 Ordination plot (MDS) of the relationship between the field data and the environmental variables (significant at $p \leq 0.05$) (stress level = 0.14).

Table 3.12 Contribution of the environmental variables to the ordination by principal axis correlation.

Environmental Variable	Symbol	r
Aluminum	Al	0.7335
Cadmium	Cd	0.8535
Chromium*	Cr	0.6061
Copper	Cu	0.7888
Iron	Fe	0.9043
Magnesium	Mg	0.8037
Manganese	Mn	0.6783
Nickel	Ni	0.8047
Lead*	Pb	0.3530
Zinc*	Zn	0.3228
Total Organic Carbon	TOC	0.7859
Silt*	Silt	0.5712
Clay*	Clay	0.4045
Sand*	Sand	0.5692

* not significant based on Monte-Carlo permutation tests ($p \leq 0.05$)

Based on the Monte-Carlo permutations, aluminum (Al), cadmium (Cd), copper (Cu), iron (Fe), nickel (Ni), magnesium (Mg), manganese (Mn), and total organic carbon (TOC) are significant ($p \leq 0.05$). The metals Cd, Cu, Ni, and Fe appear to be strongly associated with the same toxic behaviour as seen by the similar location of the vectors in ordination space (Figure 3.4). These four metal vectors are in close proximity to De, which has the highest cadmium and copper concentration, and to S4, which has the highest nickel and iron concentration (and also high cadmium and copper concentrations) (Table 3.8). A strong association with toxic behaviour also exists between Al, TOC, and Mn and Mg (vectors located orthogonal to the Cd, Ni, and Fe vectors). These vectors are associated with the Collingwood Harbour sediments, which contain the highest percent TOC and highest concentrations of Al, Mn, and Mg (Table 3.8).

Figures 3.3 and 3.4 show a relationship with the metal vectors (Ni, Fe, Cu and Cd) (upper right corner in Figure 3.4) and *Hexagenia* survival, *Tubifex* survival and *Tubifex* cocoon production (lower left corner in Figure 3.3). This corresponds with what is seen in the sediments with the highest metal concentrations. Sites De, S4 and Cu3 produced lower worm and mayfly survival, negative mayfly growth, and low or no cocoon production (Table 3.9). The TOC, Al, Mg, and Mn vectors (lower right corner in Figure 3.4) show a relationship with *Hyalella* survival and growth and *Hexagenia* growth (located in a similar location as the environmental vectors) and *Tubifex* cocoon hatch and young production (upper left corner in Figure 3.3). The site with the highest TOC, Al, Mg, and Mn (Collingwood Harbour sites) (Table 3.8) show the highest amphipod survival, the highest mayfly growth, and also show the greatest toxicity to *Tubifex* reproduction (percent cocoon hatch and young production) (Table 3.9).

To further explore the relationship between the environmental variables and the test endpoints, multiple regression was performed. The regression of each test endpoint on the environmental variables is shown in Table 3.13. Variable(s) that are significantly ($p \leq 0.05$) related to the endpoint are marked with an 'X'. A single variable to a combination of up to six variables best explains the variation in test endpoints. Two metals (lead and zinc) that were not significant variables in the Monte-Carlo tests from principal axis correlation are included in some cases (HASU and HLGW). Results from the multiple regression are in good agreement for the most part with the ordination results. For instance, TOC best explains the variation in *Tubifex* hatch (although the adjusted r^2 is low), which is in agreement with the location of TTHT and TOC (in opposite directions) in the ordination plots (Figures 3.3 and 3.4). There is a significant relationship between TOC, copper, and nickel (also lead) and *Hexagenia* growth ($r^2 = 0.885$), also in agreement with the location of these vectors in ordination space. TOC is in a similar location to HLGW, while the metal vectors are located in opposite direction. Nickel best explains the variation in both *Hexagenia* and *Tubifex* survival ($r^2 = 0.555$ and 0.405 respectively), and these vectors are located in opposite directions to each other as well (Figures 3.3 & 3.4). Six metals including cadmium, copper and nickel, and aluminum (also lead and zinc) best explain the variation in *Hyalella* survival. The aluminum vector is in close proximity to the HASU vector (lower right quadrant), while the other metal vectors are located in the opposite quadrant (upper right) to HASU. Finally, there is a significant relationship between cadmium and magnesium and *Hyalella* growth, with magnesium located in the same proximity to HLGW, while cadmium is located in the opposite direction (Figures 3.3 and 3.4).

Table 3.13 Stepwise regression of each test endpoint on environmental variables. An 'X' denotes a significant relationship ($p \leq 0.05$).

Endpoint	TOC	sand	Al	Cd	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn	Adjusted r^2
HASU			X	X		X				X	X	X	0.927
HAGW				X				X					0.742
CRSU								X	X				0.358
CRGW				X		X				X			0.949
HLSU										X			0.555
HLGW	X					X				X	X		0.885
TTSU										X			0.405
TTCC									X	X			0.565
TTHT	X												0.385
TTY						X			X				0.840

To provide some context of the severity of contamination in the field-collected sites, a comparison was made between the toxicity test responses in the field-collected sites with data from 116 reference sites. The resultant three-dimensional configuration is shown in the first two axes in Figure 3.5, in the first and third axes in Figure 3.6, and in the second and third axes in Figure 3.7. The field sites are defined as non-toxic, possibly toxic, toxic or severely toxic in relation to their similarity to the reference sites. The inner to outermost bands represent the 90%, 99%, and 99.9% probability ellipses around the 116 reference sites (not shown for clarity). Divergence from the reference sites indicates differences in the toxicity test responses of the four species from that expected. Sites that are located inside the 90% probability ellipse (band 1) are considered the same as reference and thus non-toxic. Sites located between the 90 - 99% probability ellipses (between bands 1 and 2) are considered possibly different to the reference sites and thus potentially toxic, and sites located between the 99 - 99.9% probability ellipses (between bands 2 and 3) are considered different from the reference condition and thus toxic. Finally, sites located outside the 99.9% probability ellipse (band 3) are considered very different from the reference condition and thus severely toxic.

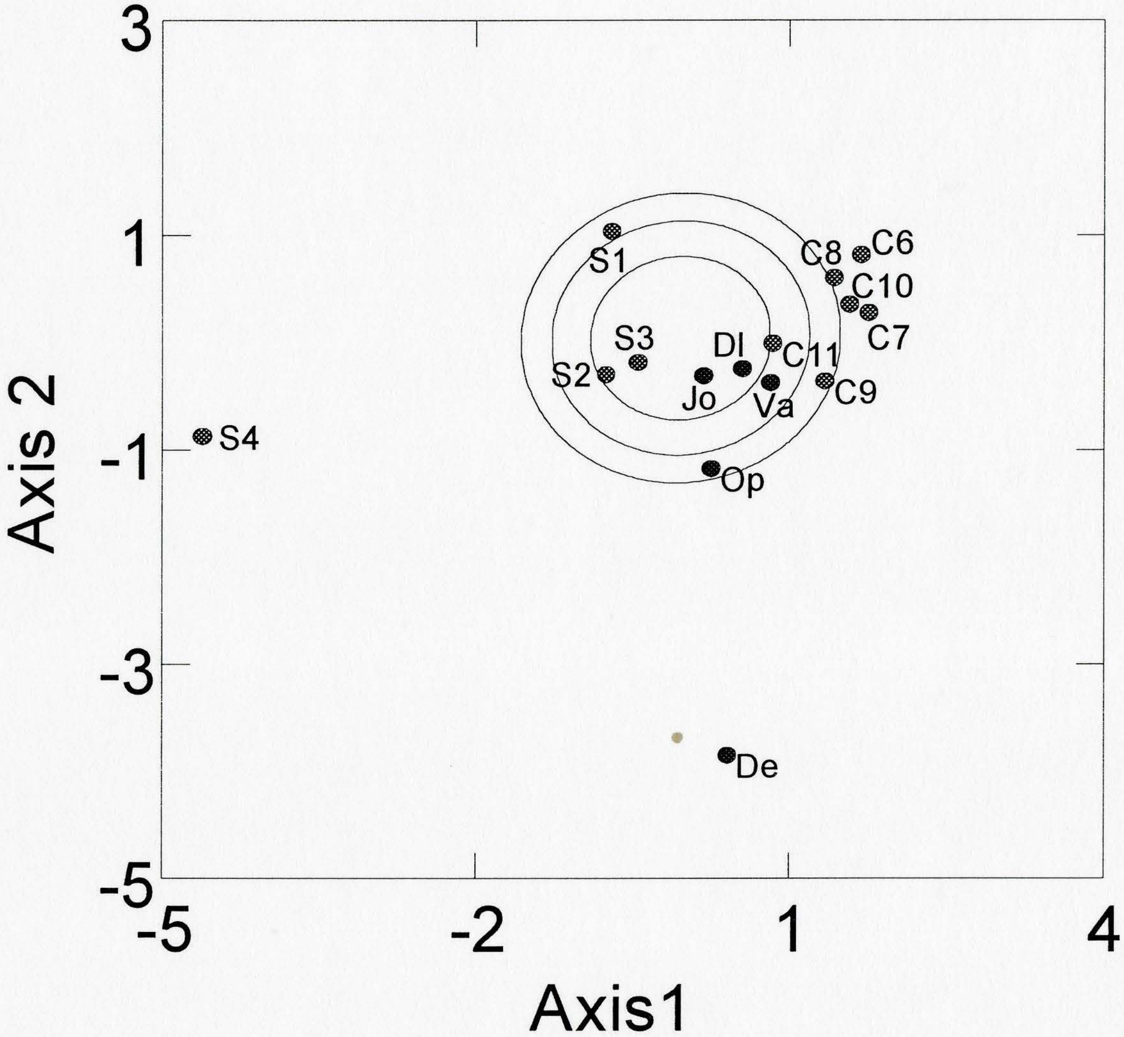

Figure 3.5 Ordination plot (MDS) of field sites relative to reference sites (not shown) in the first two axes. The inner to outermost ellipses represent the 90%, 99% and 99.9% probability ellipses around the reference sites (stress level = 0.13).

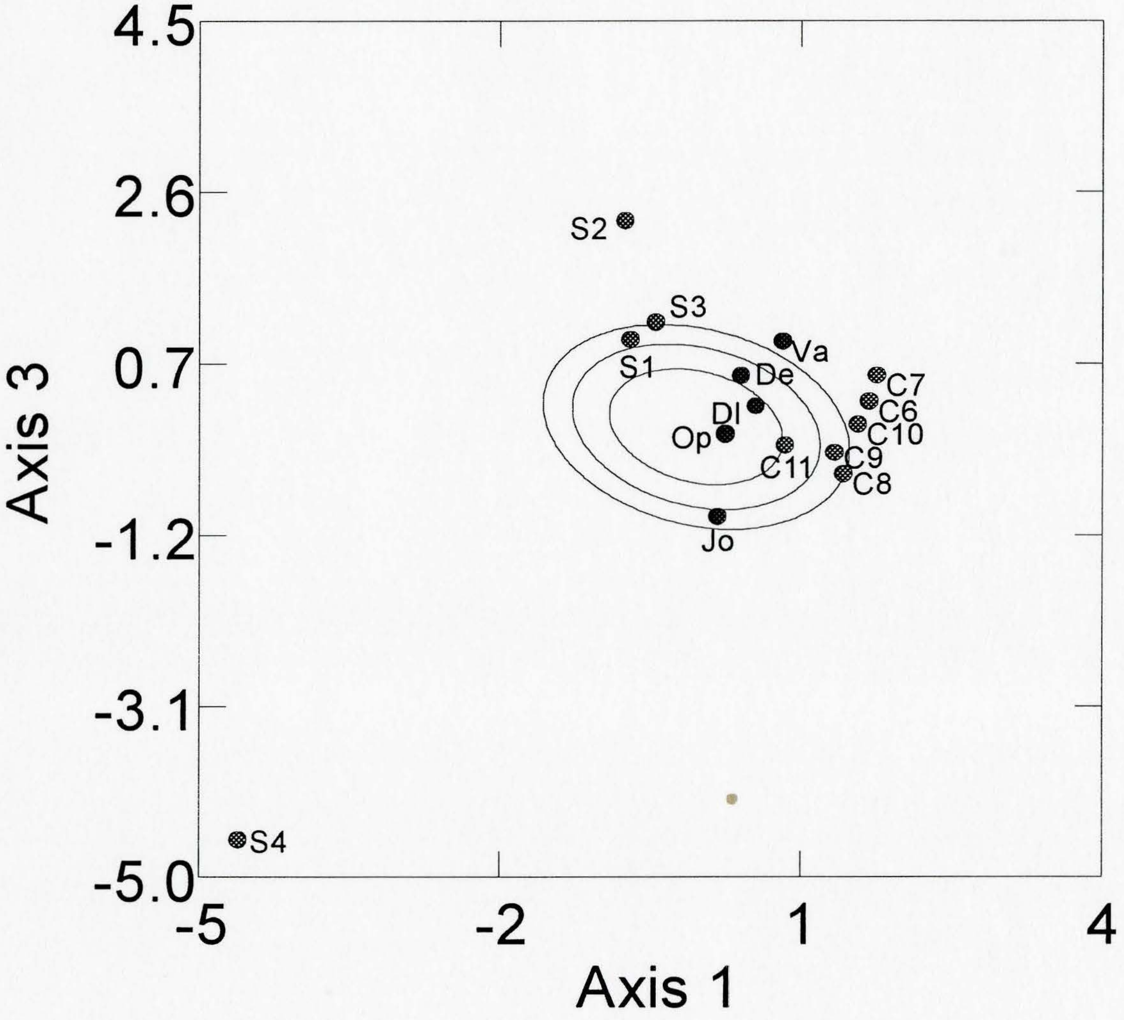

Figure 3.6 Ordination plot (MDS) of field sites relative to reference sites (not shown) in axes one and three. The inner to outermost ellipses represent the 90%, 99% and 99.9% probability ellipses around the reference sites (stress level = 0.13).

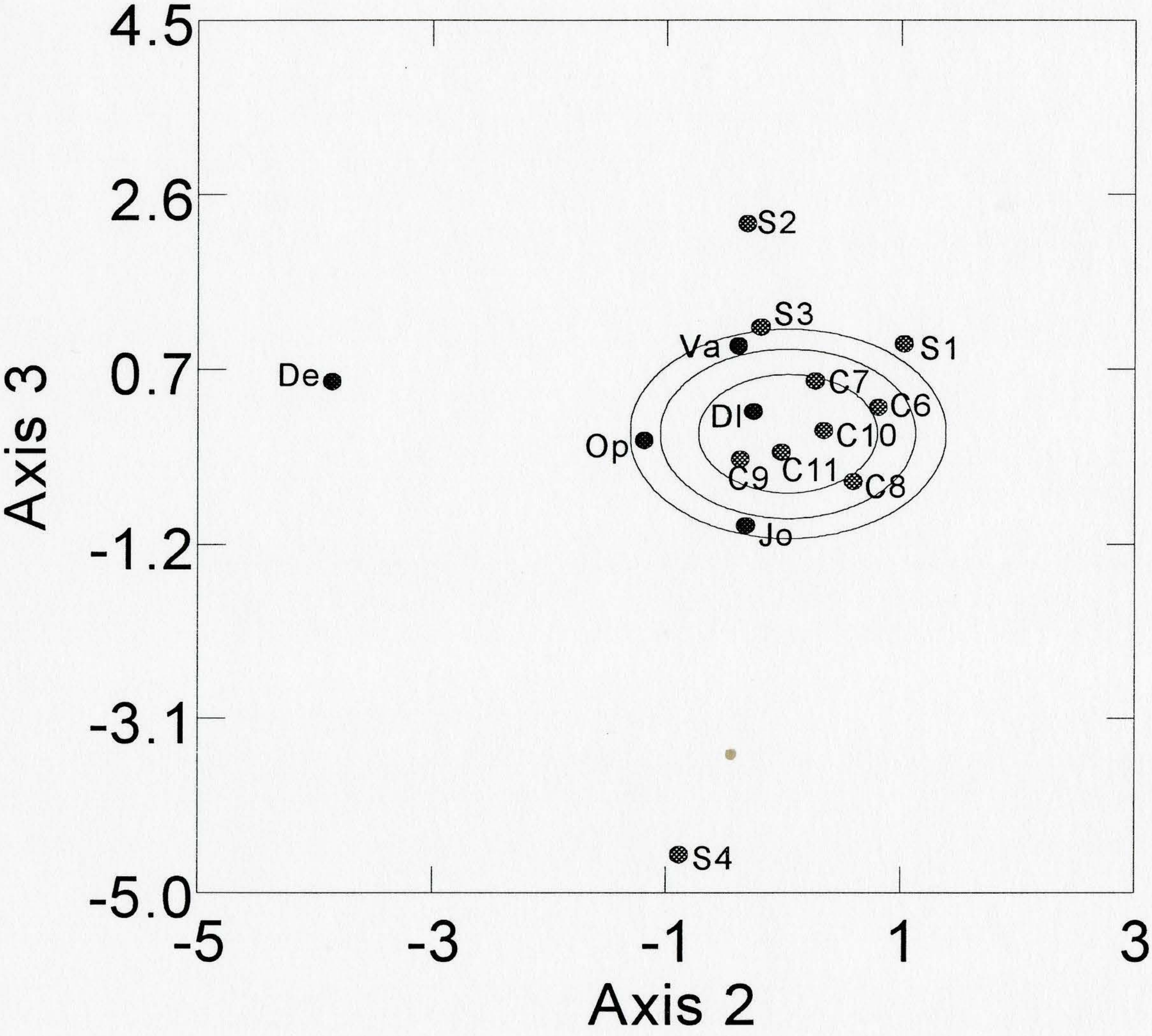

Figure 3.7 Ordination plot (MDS) of field sites relative to reference sites (not shown) in axes two and three. The inner to outermost ellipses represent the 90%, 99% and 99.9% probability ellipses around the reference sites (stress level = 0.13).

On the first two axes (Figure 3.5), six of the fifteen field sites fall into the severely toxic category, located in band 4 (outside the 99.9% probability ellipse) and include Collingwood Harbour sites C6, C7, C8, C10, Rouyn-Noranda site De, and Sudbury site S4. The sites in this category are most different to the reference sites. Three sites (Collingwood Harbour site C9, Rouyn-Noranda site Op and Sudbury site S1) fall into the toxic category, located in band 3. Two sites (Rouyn-Noranda site Va, and Collingwood Harbour site C11) fall into the potentially toxic category, located in band 2. Non-toxic sites, which are located in band 1, include Rouyn-Noranda sites Dl and Jo, and Sudbury sites S2 and S3. These last four sites are the most similar to the reference sites. Figure 3.6 (axes one and three) shows four sites in a greater toxicity category than shown by the first two axes. Sites S2, S3, and Va are located in band 4 (severely toxic), and Jo is located in band 3 (toxic). Figure 3.7 (axes 2 and 3) shows five sites in band 1 (Dl, C7, C9, C10, C11), two sites in band 2 (C6, C8), three sites in band 3 (Op, Jo, Va), and five sites in band 4 (S1, S2, S3, S4, De). A summary and overall site category is given in Table 3.11.

Table 3.14 Summary of toxicity assessment in field-collected sites in relation to data from 116 reference sites in three dimensions. Sites are categorized as non-toxic (NT), potentially toxic (PT), toxic (T) or severely toxic (ST).

	SITE			
Band	**Axes 1 - 2**	**Axes 1 - 3**	**Axes 2 - 3**	**Overall**
1 (NT)	DI, Jo, S2, S3	DI, Op	DI, C7, C9, C10, C11	DI
2 (PT)	C11, Va	C11, De,	C6, C8	C11
3 (T)	C9, Op, S1	C8, C9, S1, Jo	Op, Jo, Va	Op, Jo, C9
4 (ST)	C6, C7, C8, C10, S4, De	C6, C7, C10, S4, S2, S3, Va	S1, S2, S3, S4, De	S1, S2, S3, S4, Va, De, C6, C7, C8, C10

4. DISCUSSION

4.1 Water-Only/Sediment Exposures

4.1.1 96-hour water-only exposures

Different test conditions in water-only exposures (i.e. water hardness, pH, temperature, feeding, test duration), as well as age and condition of test organism have resulted in a wide range of LC50's reported for the same or closely related species of benthic invertebrates (Warnick and Bell 1969; Rehwoldt *et al.* 1973; Clubb *et al.* 1975; Nehring 1976; Brković-Popović and Popović 1977a; Spehar *et al.* 1978; Leonhard *et al.* 1980; Rao and Saxena 1981; Chapman *et al.* 1982; Gauss *et al.* 1985; Williams *et al.* 1985; Powlesland and George 1986; Kosalwat and Knight 1987b; Khangarot 1991; Collyard *et al.* 1994; Fargasova 1994; Phipps *et al.* 1995; Reynoldson *et al.* 1996; Suedel *et al.* 1996; Milani *et al.* 1996; Deaver and Rodgers 1996; Borgmann *et al.* 1998). A summary of the LC50's for cadmium, copper, and nickel from these studies is shown in Table 4.1.

Table 4.1 Summary of cadmium, copper and nickel LC50's (mgl^{-1}) from previous studies.

Group	Species	Exposure Period	LC50 Cadmium	LC50 Copper	LC50 Nickel	Reference
Amphipod	*Gammarus* sp.	96-h	0.07	0.91	13.0	Rehwoldt et al. (1973)
	Gammarus pulex	96-h	0.03	-	-	Williams et al. (1985)
	H. azteca	96-h	0.007-0.013	0.034-0.052	-	Collyard et al. (1994)
	H. azteca	10-d	0.0028	0.031	0.78	Phipps et al. (1995)
	H. azteca	96-h	-	0.066	-	Suedel et al. (1996)
	H. azteca	96-h	-	0.176	-	Milani et al. (1996)
	H. azteca	10-d	-	0.042-0.014	-	Deaver and Rodgers (1996)
	H. azteca	1-wk	0.008	0.139	0.079	Borgmann et al. (1998)
	H. azteca	96-h	0.013	0.21	3.62	Present study
Midge	*Chironomus* sp.	96-h	1.2	0.03	8.6	Rehwoldt et al. (1973)
	Chironomus sp.	48-h	25.0	-	-	Rao and Saxena (1981)
	C. riparius	96-h	300	-	-	Williams et al. (1985)
	C. tentans	96-h	-	0.037	-	Gauss et al. (1985)
	C. riparius	24-h	2.1	-	-	Williams et al. (1986)
	C. riparius	48-h	-	-	79.5	Powlesland and George (1986)
	C. decorus	48-h	-	0.739	-	Kosalwat and Knight (1987b)
	C. tentans	10-d	-	0.054	-	Phipps et al. (1995)
	C. tentans	96-h	-	0.63	-	Suedel et al. (1996)
	C. riparius	96-h	-	0.861	-	Milani et al. (1996)
	C. riparius	96-h	0.021	0.043	5.25	Present study

h = hour, d = day, wk = week

Table 4.1 continued.

Group	**Species**	**Exposure Period**	**LC50 Cadmium**	**LC50 Copper**	**LC50 Nickel**	**Reference**
Mayfly	*E. subvaria*	96-h	2.0	(48-h) 0.32	4.0	Warnick and Bell (1969)
	E. grandis	96-h	28.0	-	-	Clubb et al. (1975)
	E. grandis	14-d	-	0.18-0.20	-	Nehring (1976)
	Ephemerella sp.	28-d	<0.003	-	-	Spehar et al. (1978)
	Hexagenia rigida	96-h	6.2	-	-	Leonard et al. (1980)
	E. ignita	96-h	13.0	-	-	Williams et al. (1985)
	B. rhodani	96-h	0.5	-	-	Williams et al. (1985)
	Hexagenia spp.	96-h	7.82	0.073	75.96	Present study
Tubificid	*T. tubifex*	48-h	0.72	0.89	61.4	Brković-Popović and Popović (1977a)
	T. tubifex	96-h	0.32	-	-	Chapman et al. (1982)
	L. hoffmeisteri	96-h	0.17	-	-	Chapman et al. (1982)
	L. hoffmeisteri	96-h	2.9	-	-	Williams et al. (1985)
	T. tubifex	96-h	47.53	0.158	66.75	Khangarot (1991)
	T. tubifex	96-h	1.03	-	-	Fargasova (1994)
	T. tubifex	96-h	3.2	0.09	-	Reynoldson et al. (1996)
	T. tubifex	96-h	0.87	0.16	17.64	Present study
Naidid	*Nais* sp.	96-h	1.7	0.09	14.1	Rehwoldt et al. (1973)
Lumbriculid	*L. variegatus*	10-d	0.158	0.035	12.16	Phipps et al. (1995)

h = hour, d = day, wk = week

Including the present study, LC50's for cadmium range from 0.0028 to 0.07 mg/L for amphipods, from 0.021 to 300 mg/L for midges, from <0.003 to 28 mg/L for mayflies, and from 0.17 to 47.5 mg/L for tubificids. For copper, the range in LC50's are from 0.031 to 0.91 mg/L for amphipods, from 0.03 to 0.86 mg/L for midges, from 0.073 to 0.32 mg/L for mayflies, and from 0.09 to 0.89 mg/L for tubificids. For nickel, the range in LC50's are from 0.08 to 13 mg/L for amphipods, from 5 to 80 mg/L for midges, from 4 to 76 mg/L for mayflies, and from 18 to 67 mg/L for tubificids (Table 4.1). The lack of uniformity in test conditions makes valid comparisons difficult, however some points should be noted. First, members of the same taxonomic groups can show variation in sensitivity. For example, Williams *et al.* (1985) found two species of mayflies, *Ephemerella ignita* and *Baetis rhodani* to have 96-hour LC50's for cadmium of 0.5 and 13.0 mg/L respectively. Among nine species of oligochaetes, Chapman *et al.* (1982) found a range in 96-hour cadmium LC50's of 0.17 - 0.63 mg/L. Wiederholm *et al.* (1987) reported differences in tolerance to copper in spiked sediment between *T. tubifex* and *L. hoffmeisteri*, with lower growth and reproduction exhibited by *T. tubifex*. Second, the same species from different populations show different sensitivity. Reynoldson *et al.* (1996) found a 1.3 - 2.5-fold difference in 96-hour LC50's for a Canadian and a Spanish population of *T. tubifex* exposed to cadmium, copper and chromium. The two cultures represent populations from a lentic (Canadian) and lotic (Spanish) environment and therefore may be adapted to different environmental conditions. The

Spanish population was found to be less tolerant for all metals, and the authors attributed these differences to genetic variability derived from different natural populations. Looking at mortality and feeding rate in amphipods, Maltby and Crane (1994) found differences in sensitivity between two populations of *G. pulex* exposed to toxicants. One population was collected from an uncontaminated stream (less tolerant) and the other from a metal-contaminated site (more tolerant). They attributed possible difference in sensitivity to the development of metal tolerances by physiological acclimation (the same two populations did not show the same degree of difference in laboratory tests conducted 18 months later).

Regardless of the noted differences in sensitivity exhibited between similar species or between different populations of the same species, comparing the overall order in sensitivity for each metal between the groups of animals reveals similar findings between studies. In general, amphipods tend to be more sensitive than chironomids, which in turn were found to be more sensitive than oligochaetes. For example, examining the metal sensitivities of an oligochaete (*Nais sp.*), an amphipod (*Gammarus sp.*), and a midge (*Chironomus sp.*) in 96-hour exposures, Rehwoldt *et al.* (1973) found the order in increasing LC50's to cadmium to be *Gammarus sp.* < *Chironomus sp.* < *Nais sp.*, with LC50's of 0.07, 1.2, and 1.7 mg/L respectively (Table 3.2). For copper, the order in increasing LC50's was *Chironomus sp.* < *Nais sp.* < *Gammarus sp.*, with LC50's of 0.03, 0.09, and 0.91 mg/L respectively (Table 3.2). The order in species sensitivity for both metals in this 1973 study is consistent to findings in this study. The order in increasing LC50's to nickel (midge (8.6 mg/L) < amphipod (13.0 mg/L) < oligochaete (14.1 mg/L)) (Table 3.2) do not follow the same order as that observed in the present study (amphipod ≤ midge < oligochaete). However, the nickel LC50's for *C. riparius* and *H. azteca* in the present study are similar (overlapping ranges) (Table 3.1).

Studies on mayfly metal-sensitivities were found to be less frequent than the above mentioned groups (Table 4.1). Williams *et al.* (1985), found the mayflies *E. ignita* and *B. rhodani* to be less sensitive than the amphipod *Gammarus pulex* to cadmium, and *B. rhodani* to be more sensitive than the tubificid, *L. hoffmeisteri*, while *E. ignita* was not more sensitive than *L. hoffmeisteri* (Table 4.1). Both mayflies were more sensitive than *C. riparius* to cadmium, which is not consistent with the findings in the present study. However, Williams *et al.* (1985) used a later chironomid instar stage (10-12 mm), which is known to be considerably less sensitive than the first instar stage (Gauss *et al.* 1985; Williams *et al.* 1986; Timmermans *et al.* 1992).

Metal-specific sensitivities in the same or similar species in other studies generally agree with the present study. For example, in examining *T. tubifex* sensitivity to metals in 96-hour exposures, Khangarot (1991) found copper more toxic to *T. tubifex*, followed by cadmium and nickel, with LC50's of 0.158, 47.53, and 66.75 mg/L respectively. Reynoldson *et al.* (1996) also found copper more toxic than cadmium to *T. tubifex*, with LC50's of 0.09 and 3.2 mg/L respectively.

Warnick and Bell (1969) found copper most toxic to the mayfly *E. subvaria*, followed by cadmium and nickel with LC50's of 0.32, 2.0, and 4.0 mg l^{-1} respectively in 48- or 96-hour exposures. In 10-day exposures, Phipps *et al.* (1995) found *H. azteca* more sensitive to cadmium, followed by copper and nickel with LC50's of 0.0028, 0.031, and 0.78 mg l^{-1} respectively, and *L. variegatus* more sensitive to copper followed by cadmium and nickel, with LC50's of 0.04, 0.16, and 12.2 mg Cd l^{-1} respectively. Finally, in 96-hour exposures examining amphipod sensitivity to metals, Rehwoldt *et al.* (1973) found cadmium to be more toxic to *Gammarus sp.*, followed by copper and nickel with LC50's of 0.07, 0.91, and 13.0 mg l^{-1} respectively. Differences in metal-specific sensitivities also exist, however. For instance Brković-Popović and Popović (1977a) found cadmium more toxic to *T. tubifex,* followed by copper, and nickel in 48-hour exposures, with LC50's of 0.72, 0.89, and 61.4 mg l^{-1} respectively. Borgmann *et al.* (1998) found *H. azteca* most sensitive to cadmium, but found the amphipods more sensitive to nickel than copper in 1-week exposures, with LC50's of 0.008, 0.079, and 0.139 mg l^{-1} respectively. Rehwoldt *et al.* (1973) found *Chironomus* sp. more sensitive to copper followed by cadmium and nickel, with LC50's of 0.03, 1.2, and 8.6 mg l^{-1} respectively.

In the present study, differential sensitivity to cadmium, nickel, and copper are exhibited by the four species in the water-only exposures. While the order in metal sensitivity between the species is in good agreement with previous studies examining metal sensitivity in the same or closely related species, actual LC50's are not because of the above-mentioned test shortcomings (various test conditions employed, age of organism, etc.). In summary, *Hyalella* is the most sensitive species to both cadmium and nickel, and *Chironomus* is the most sensitive species to copper. *Hexagenia* exhibits the greatest range in LC50's (1041-fold range) across the three metals in the water-only exposures, appears to be relatively intolerant to cadmium and nickel compared to the other species, yet is sensitive to copper. It should be noted however, that the nickel LC50 for *Hexagenia* might not be reliable because of the decrease in water hardness in the higher concentrations. The detoxification mechanisms of water hardness on metal toxicity are well known (Borgmann 1983; Stephenson 1983; Wang 1987). The decrease in calcium and magnesium ions as a result of the dilution of the culture water with stock solution in the *Hexagenia* nickel series may have lead to a decrease in competition between nickel and these cations, subsequently increasing toxicity. The reported LC50 may then be an underestimation of the LC50, although other factors generally accompanying a reduction in hardness (i.e. lowering of pH) may also influence toxicity.

Different mechanisms of toxicity between species and between the three metals likely exist (discussed further below) as there appears to be no single mode or mechanism of toxicity for all metals due to their elemental nature and their varied affinities for organic ligands in biological structures (Mason and Jenkins 1995). The ability to cope with the metal (i.e. sequestering of the metal in granules; detoxifying mechanisms such as specific metal binding proteins) is also highly species-dependent (Langston and Spence 1995), resulting in varied metal accumulation and toxicity observed between species.

4.1.2 Spiked-sediment exposures

The results from spiked-sediment tests indicate that the relative sensitivities of the four species are not accurately predicted from the 96-hour water-only exposures. *Hexagenia* is less sensitive than *Tubifex* to cadmium and nickel in the water-only exposures yet the two show similar sensitivity or *Hexagenia* shows greater sensitivity to cadmium and nickel in the spiked-sediment tests (Tables 3.2, 3.3, & 3.4). *Chironomus* is the most sensitive organism to copper in the water-only exposures but is less sensitive than both *Hyalella* and *Hexagenia* to copper in the spiked sediment exposures (Tables 3.1,3.2, 3.3, & 3.4). *Chironomus* and *Hyalella* also show comparable sensitivity to nickel in the water-only exposures, but *Chironomus* is far less sensitive than *Hyalella*, (a ≥10-fold difference in LC50's in all test fractions) in the spiked sediment exposures (Tables 3.2, 3.3, & 3.4). Finally, *Hyalella* is the least sensitive organism to copper in the 96-hour water-only exposures (Table 3.1), yet is the second most sensitive after *Hexagenia* in the spiked sediment tests (Tables 3.2, 3.3, & 3.4).

Others (West *et al.* 1993) have also found differences in the order of species sensitivity in water-only versus sediment exposures. In determining the relative sensitivities of *H. azteca*, *C. tentans*, and *L. variegatus* in 10-day water-only exposures and in copper contaminated sediments from the Keweenaw waterway, West *et al.* (1993) found similar LC50's for *L. variegatus* and *H. azteca* in the waterborne tests (35 and 31 µg/L respectively). Exposure in field-collected sediments, however, resulted in reduced survival in *Hyalella* in 8 of 11 sites while survival was unaffected for *L. variegatus* in all sites. Additionally, *Chironomus* survival was reduced in 7 of the 11 field sites, yet in the waterborne exposures, the LC50 was higher than the other two species (54 µg/L), suggesting exposure route differences and possible geochemical influences of the sediment (West *et al.* 1993).

Endpoint Sensitivity

A comparison of LC25's and IC25's shows *Hyalella* survival and growth to be similarly sensitive indicators of chronic toxicity for the three metals tested. Similar results were found by Borgmann *et al.* (1989) whom reported that cadmium concentrations that did not cause mortality also did not cause a growth effect in *Hyalella*. This may indicate that for *Hyalella*, the mode of toxicity for these three metals may be the same and that survival may be sufficient to infer cadmium, copper, and nickel toxicity.

Chironomus growth is more sensitive endpoint than survival for all metals as evidenced by the lower IC25's, yet in the dissolved phases, LC25's and IC25's are nonetheless more similar for cadmium and copper than for nickel. That nickel is the least sensitive metal to *Chironomus* (as well as for the other three species) in both exposure types (water-only and spiked-sediment tests) suggests that the specific nature of the metal is important in explaining species sensitivities. For instance, while copper, cadmium, and nickel are classed as borderline metals, copper and cadmium also exhibit characteristics of group B metals (highly reactive, lack of specific binding to organic ligands, form strong covalent bonds) and group B metals tend to be persistent and toxic in small amounts (Mason and Jenkins 1995).

Hexagenia growth is more sensitive than survival for all the metals tested. Copper, however, exerts a greater affect on *Hexagenia* survival than the other two metals. *Hexagenia* growth is similarly sensitive to *Hyalella* growth for cadmium (ranges overlap). Day *et al.* (1998) also found *Hexagenia* survival and growth a sensitive indicator of chronic toxicity (more sensitive than *Hyalella*), reporting a greater than 2-fold difference in IC25's between *Hyalella* and *Hexagenia* in tributytin-spiked sediment. Why copper exerts a stronger effect on the mayfly may be due to differences in the localization of each metal (discussed further below).

Overall, *Tubifex* reproduction is a more sensitive endpoint than survival for all metals and the number of young produced per adult is the most sensitive reproduction endpoint. Each endpoint is important in explaining specific metal effects. For instance, a decrease in cocoon production from the control group or low cocoon production (compared to a reference condition) may indicate an effect on gamete formation (gametogenesis) inside the worm. Hatching success of the cocoons is an indication of cocoon viability. Low hatching success indicates an effect on embryo development (embryogenesis) inside the cocoon. Caution should be taken in interpreting hatching success in highly toxic sediment since a high percentage of cocoons may hatch, but with a very small number of cocoons produced. Since the test is initiated with sexually mature organisms, already formed cocoons may be released quickly into contaminated sediment and subsequently hatch. Another important point is that young reproduction is the most variable endpoint tested. Cocoon production, though not as sensitive as young production, is less variable therefore may be better at discriminating between clean and contaminated sites (Reynoldson 1994).

Tubifex were quite tolerant of all the metals tested in sediment as shown by the high chronic LC25's. Compared to the growth effects of the other three species, reproduction appears to be an insensitive indicator of chronic toxicity. It is possible that spiked sediment exposures provide the most bioavailable form of a metal (i.e. dissolved forms) to the organisms. Therefore, organisms in which the dissolved form of the metal are likely the primary exposure (i.e. *Hyalella*, *Chironomus* and perhaps *Hexagenia*) will be more affected than an organism that acquires metal from the ingestion of particles (i.e. *Tubifex*). Thus, the spiked tests may not be providing an accurate estimate of metal sensitivity to *Tubifex*. Nonetheless, *Tubifex* reproduction is documented to be a more sensitive endpoint than survival, and a sensitive indicator of chronic toxicity (Wiederholm *et al.* 1987; Reynoldson *et al.* 1991). Others (Reynoldson and Day 1994; Reynoldson and Day 1998a) have reported cases where *Tubifex* was the most sensitive of the four species tested in contaminated sediments (although the causative agent(s) of toxicity were not specifically identified). The differential response to metal toxicity exhibited by the species, and the fact that *Tubifex* is likely the only organism acquiring metal from the solid phase (including pore water) stresses the importance of using *Tubifex* in the evaluation of sediment toxicity.

Several possible reasons exist (in addition to the previously mentioned metal sequestering and detoxification abilities of the organism and chemical nature of the metal) for the observed difference in the order of species sensitivity between water-only and spiked sediment tests, and for the observed difference in sensitivity between the four species for the three metals. These include exposure time, metal uptake routes and internal distribution of the metal, bioturbation, test conditions, and other factors (physiological requirements and metal regulation).

1) Exposure Time

Reasons for the differences in the order of species sensitivities observed between the two exposure types may be due to exposure time. Thorp and Lake (1974) have suggested that a 96-hour exposure period is not sufficient to reflect the true acute toxicity for invertebrates. Clubb *et al.* (1975) found that lethal toxicity to cadmium continued after 96-hours for the mayfly *Ephemerella grandis grandis*, with the 96-hour LC50 decreasing from 28 mg/L to 17.5 mg/L by day 10. Spehar *et al.* (1978) reported that in a 28-day water-only exposure, the mayfly

Ephemerella sp. experienced greater than 50% mortality not until the last week of exposure. This appears to be an important consideration for *Hexagenia* (for cadmium and nickel) as the mayfly is less sensitive than *Tubifex* to the two metals in the acute tests yet more sensitive than *Tubifex* in the chronic tests. This may also explain why *Hyalella* is the least sensitive organism to copper in the acute tests and one of the most sensitive organisms in the chronic tests. Thus, certain metals may be slower acting poisons to species (i.e. copper for *H. azteca* and cadmium and nickel for *Hexagenia* spp.), and therefore, a longer exposure period may have been warranted.

2) Uptake Routes

The four species exhibit different lifestyle and feeding behaviours that may result in different uptake routes, either through the dissolved phase or through particle ingestion. In the water-only tests, the exposure route is across the body surface or gut wall, and/or across the gills for amphipods and mayflies. In the spiked sediment tests, exposure through ingested sediment particles may play a more important role for certain organisms than others. Internal distributions of metals may also differ between species explaining the observed difference in sensitivity between the species (and may also explain differences in metal sensitivity within the same species) (Hare *et al.* 1991; Rainbow and Dallinger 1993).

a) H. azteca

Hyalella is an epibenthic species, and although it feeds off the bottom sediments, it remains at the surface of the sediment generally in the oxic layer and is often seen swimming in the water column. *Hyalella* is the least sensitive organism to copper in the water-only tests, yet one of the most sensitive in the spiked sediment tests. It is unlikely that *Hyalella* assimilate more metal from the bulk sediment and/or pore water fractions than the other species, since by nature the other organisms (i.e. *Tubifex* and *Hexagenia*) likely ingest more sediment particles than *Hyalella.* In fact, studies have shown that *Hyalella* assimilate cadmium from the overlying water fraction, not the sediment fraction (bulk sediment and pore water) (Suedel *et al.* 1996; Hare and Tessier 1998; Warren *et al.* 1998). Cairns *et al.* (1984) also found little, if any, toxicity attributed to sediment bound copper for *H. azteca* in their 10-day spiked sediment tests, and found that soluble copper best explained the observed toxicity. Deaver and Rodgers (1996) found toxicity to *H. azteca* was better related to total dissolved copper in the overlying water than by sediment bound copper in 10-d spiked sediment exposures, although toxicity was best explained by the bioavailable fraction of the dissolved metal. Borgmann and Norwood (1999) found that there was no difference in lead accumulation in *H. azteca* exposed to lead-spiked sediment and exposed in the same test beakers in cages hanging above the sediment, indicating that *Hyalella* assimilate dissolved metal from the soluble fraction. Borgmann and Norwood (1997) reported a 4-week

LC25 for copper in waterborne tests for *H. azteca* of 0.021 mg/L, which is close to the overlying water LC25 for copper in the present study (0.012 mg/L), suggesting that the dissolved phase is the likely exposure route for *Hyalella*. Therefore, given its lifestyle and the previous work described, *Hyalella* most likely acquire metal from the dissolved phase which is generally more efficient than direct uptake from particulates (Luoma 1989). This in part may explain why *Hyalella* is generally one of the more sensitive species to metal toxicity. However, this does not explain *Hyalella*'s response to copper. As previously mentioned, copper may be a slower acting poison for *Hyalella*.

b) C. riparius

Chironomus build open-ended cases in the upper oxic layer of the sediment, and their rhythmic body motions continually filter water and particles through the case (Pennak 1953). Thus, *Chironomus*, like *Hyalella*, may also acquire most of the metal in the dissolved form from the water column. Craig *et al.* (1998) found in both 5-day water-only exposures and 140-day sediment exposures that cadmium localized in the gut in *Chironomus staegeri*, suggesting that the cadmium source may be through the dissolved phase under both exposure regimes. Warren *et al.* 1998 found that the midge *Chaoborus punctipennis* accumulated 99.7% of cadmium from the overlying water, not responding to a cadmium gradient in sediment. Given *Chironomus*'s likely metal accumulation from the more efficient dissolved phase and since *Chironomus* is less sensitive than *Hyalella*, this may suggest that uptake sites (i.e. *Hyalella* gills may be a more efficient uptake site) as well as species-specific detoxification mechanisms differ between the two organisms.

c) *Hexagenia* spp. and *T. Tubifex*

Hexagenia and *Tubifex* burrow into deeper layers of the sediment, and this behaviour as well as the depth to which they burrow may affect exposure and metal accumulation (Hare 1992). The mayfly constructs a U-shaped burrow, and filters water continually through this tube (Pennak 1953), thereby remaining in constant contact with dissolved forms of metal in the water column. By modelling cadmium accumulation in the mayfly *Hexagenia limbata* in situ, Warren *et al.* (1998) found that the overlying water contributed to 97% of the total cadmium intake in the mayfly (alternatively they found tubificids accumulate 100% from the bulk sediment). However, Warren *et al.* (1998) hypothesized that this may be an overestimate due to the movement of the mayflies in and out of the treatment containers. *Hexagenia* ingest a large amount of sediment and detritus (Giesy and Hoke 1989; Warren *et al.* 1998) and since the sediment represents a more concentrated form of the metal, there is potential release of contaminants in the gut (Giesy and Hoke 1989). In examining internal distribution patterns of metals in *Hexagenia,* Hare *et al.* (1991) found differences in the localization of different metals. For example, cadmium was found mainly in the gut of the mayfly, while copper was found to a greater extent in the body and gills of the animal, suggesting food ingestion is more important for cadmium, while water may be an important source of copper. This in part may help to explain the differences in metal sensitivities in the present study (i.e. why copper is more toxic to the mayfly survival than cadmium and nickel). Timmermans *et al.* (1992) found differences in uptake between metals in the water mite (*Limnesia maculata*) and the caddisfly (*Mystacides* spp.). When exposed to both the essential metal zinc and the non-essential metal cadmium for a four-week period either via contaminated diet or through an aqueous source, they found that cadmium uptake through food dominated; yet for zinc, uptake through water dominated in both species. Thus, cadmium, copper, and nickel may target different sites within the organism, which may help explain differences in sensitivities observed between the three metals. One would also expect larger differences in water-only vs. sediment sensitivity for organisms that acquire metal from the solid phase. This is in fact what is observed in the present study with the largest differences in sensitivities between exposure types noted for *Hexagenia* and *Tubifex*. *Tubifex* may ingest particulates from deeper (anoxic) layers of the sediment than the other species and consequently is subjected to different metal fractions (i.e. association with sulfidic fraction). This fraction may be less available to the organism (Kersten and Forstner 1987), explaining *Tubifex*'s insensitivity in the spiked sediment exposures.

3) Bioturbation

An important consideration in metal uptake in sediment exposures is the role of bioturbation. Bioturbation is known to alter sediment chemistry by the upward movement of material and the release of metals from the sediment into the pore water and/or overlying water interface (Davis *et al.* 1975; Lawrence *et al.* 1982; Krantzberg and Stokes 1985; Matisoff *et al.* 1985,1995; Reynoldson 1987; Campbell *et al.* 1988; Saouter *et al.* 1993; Peterson *et al.* 1996). Peterson *et al.* (1996) showed that bioturbation by *L. variegatus* led to a reduction of AVS concentrations (by oxidation) in the surficial layer of spiked sediment and subsequently increased the metal concentration in the pore water, thereby enhancing the cadmium bioavailability. During a 9-day exposure to mercury in sediments, Saouter *et al.* (1993) reported release of mercury from diffusion as well as from bioturbation resulting from mayfly activity. Of the four species, the mayfly appears to disturb the greatest amount of sediment, judging visually by the turbidity of the overlying water in the in the test beakers. This ‘clouding’ of the overlying water would typically occur in a week or less from test initiation in fine-grained sediments. This may help to explain in part why *Hexagenia* is less tolerant than *Tubifex* to cadmium and nickel in the sediment exposures (but more tolerant in the water-only exposures). *Hexagenia* may be exposed to more toxic forms of the metals due to bioturbation and subsequent release of metals into the water phases. *Tubifex* build tubes in the sediment and ingest a large quantity of sediment (Pennak 1953). The worm’s posterior end is often seen sticking out of the mud often in a waving motion for respiratory purposes, but the tube itself is not irrigated with oxic water (Matisoff 1995). While the sediment contains the most concentrated source of metal, assimilation from this source may be inefficient (Luoma 1989). Generally, uptake from solution is more efficient than direct uptake from particulates (Luoma 1989). Benthic organisms are exposed to all forms of each metal that occur in overlying and pore water, and the speciation and total metal concentrations are usually different between pore waters and overlying water because of the more intimate contact of pore waters with sediments (Luoma 1989). In the present study, the overlying water and pore water metal concentrations were determined before the addition of the organisms, thus potential releases into the pore water and overlying water are not known. Post-test analysis of overlying and pore waters would have been useful to determine release of metal into the dissolved phases thus providing evidence of the extent of bioturbation by each species.

4) Test Conditions

The test regimes were not identical in all four tests, which may help explain observed differences in species sensitivity. Although the ratio of overlying water to sediment is identical in all four tests, more sediment was present in the *Hexagenia* and *Tubifex* tests (100 mL and 300 mL respectively vs. 75 mL for *Hyalella* and *Chironomus* tests), as *Tubifex* and *Hexagenia* are infaunal organisms and therefore require more room to burrow. The increased amount of sediment potentially allows for deeper burrowing and as such *Hexagenia* and *Tubifex* may be exposed to anoxic layers of the sediment whereas it is unlikely that *Chironomus* and *Hyalella* are. By burrowing deeper *Tubifex* and *Hexagenia* may be exposed to different forms of metal (i.e. in the subsurface sediment, the metal may be in the sulfide form) which can affect metal uptake.

The quantity and quality of supplemented food used in each of the four tests also varies. For *Hyalella and* *Chironomus*, food (8 mg crushed fish flake per feeding) is added on the surface of the sediment. For *Hexagenia*, the food is also added as described above but the diet is different (50 mg diet consisting of Nutrafin® fish flakes, Cerophyll™, and yeast per feeding). For *Tubifex*, food (80 mg fish flake) is added once (one week before the introduction of the worms) and is mixed directly into the sediment. The presence or abundance of food may affect feeding rates and rates of ingestion (Luoma 1983). Metals may adsorb to the supplemented food altering metal availability to the organisms, therefore the amount of food added, its composition, as well as the chemical of interest are important factors to consider (USEPA 1994).

It is possible that a food source (microflora and microfauna) for *Hyalella* may be eliminated by the exposure to copper, which in turn may have lead to reduced feeding by *Hyalella* and possible death through starvation. Amphipods respond to microflora species composition or the nature of organic substances in the mud (Hargave 1972). Sediment manipulation studies performed by Day *et al.* (1995b) revealed that different sediment manipulations (freezing, irradiating & autoclaving) lead to decreases in survival of *H. azteca* in both an uncontaminated sediment from Long Point, Lake Erie, and a contaminated sediment from Hamilton Harbour. This response was not observed, however, for either *C. riparius* or *T. tubifex*, and suggested the possibility that the amphipod's food source may have been affected by the manipulation.

Differences in copper and nickel sensitivities observed for *C. riparius* in the water-only versus the spiked sediment tests may be because the chironomids did not survive well in the water-only tests. Despite the use of a substrate and added food, low control survival was a problem that necessitated the repeating of the water-only tests several times. Also, the use of first instar organisms made it difficult to recover the animals after 96-hours due to their small size. Control survival was not a problem in the spiked-sediment tests run over 10 days. It is possible that abrasion by silica sand on the first instar chironomids may have lead to an increase in mortality, also reported by others (Thornton and Wilhm 1975). Pascoe *et al.* (1989) also reported high mortality in first instar *C. riparius* in their control treatment in which shredded filter paper was used as a substrate. If *Chironomus* experienced higher mortality in exposure treatments using aquarium gravel as a substrate, the LC50's may be underestimated.

5) Other factors (physiological requirements, metal regulation)

The physiological requirements of the species may dictate the amount of metal that is taken up. For instance, the similarity between cadmium and calcium ions may lead to the uptake of cadmium accidentally through calcium ion pumps across the cell membrane (Wright 1980), an important factor for crustacean species, and may explain why cadmium is the most toxic of the metals tested to *Hyalella.* Since calcium is required in relatively large amounts (Mason and Jenkins 1995) cadmium may be transported into the membrane in larger amounts than copper and nickel which are required in trace or ultra trace amounts (Mason and Jenkins 1995). Crustaceans do not appear to be able to regulate the body concentrations of non-essential metals such as cadmium (Rainbow and White 1989; Weeks and Rainbow 1991); however, the regulation of essential metals such as copper by amphipods has been reported (Borgmann *et al.* 1993; Borgmann and Norwood 1995). Rainbow and White (1989), however, did not find evidence of copper regulation by the amphipod, *Echinogammarus pirloti* after 28-day waterborne exposure to a range of copper concentrations.

Cadmium uptake through calcium channels may also occur for insect species as well. Observations by Craig *et al.* (1999) provide strong evidence that cadmium enters the midgut (the site of nutrient absorption) of *Chironomus staegeri* via calcium channels, and this may also help to explain why *C. riparius* is more sensitive to cadmium of the metals tested in the spiked sediment exposures in the present study.

Diagnostic capabilities of species relative sensitivities

As contaminated field sediment is often comprised of a mixture of metals, a method to discriminate between specific metal toxicity would be useful. This relative measure is achieved by taking the geometric mean of the spiked-sediment LC25's and IC25's across the four species for each metal individually.

For *T. tubifex*, only the most sensitive reproduction endpoint was used (number of young produced per adult) in the calculation. For example, the geometric mean for cadmium is taken from the individual LC25's and IC25's from Tables 3.2 and 3.5 (21 + 28 + 560 + 600 + 10 + 16 + 14 + 301 = 58.29). Individual endpoint LC25's and IC25's are then divided by this geometric mean to give the values listed in Table 4.2. For example, the LC25 for *Hyalella*, (21), is divided by 58.29 to give 0.36. These values are also presented graphically in Figure 4.1. Values below 1 indicate the more sensitive endpoints (the smaller the number the more sensitive the endpoint), and numbers > 1 indicate the least sensitive endpoints to each metal. While this was done for only the bulk sediment fraction, this can also be applied to the overlying water and pore water LC25's and IC25's. From Table 4.2 and Figure 4.1, it is evident that *C. riparius* and *Hexagenia* spp. mortality are the only discriminatory endpoints across the three metals, as the endpoint ranges span from < 1 to > 2. How the endpoint sensitivities can be applied to discriminating between metal toxicity in field sediment is shown below in three scenarios.

Table 4.2 Individual bulk sediment LC25's and IC25's for each species divided by the geometric mean of all endpoints for each metal. The range in endpoint values across the three metals is also shown.

Metal	***H. azteca***		***C. riparius***		***Hexagenia spp.***		***T. tubifex***	
	LC25	IC25	LC25	IC25	LC25	IC25	LC25	IC25
Cadmium	**0.36***	0.17	0.48*	0.27	9.61	0.24	10.29	5.16
Nickel	**0.26**	0.22	2.72	0.79	1.74	0.45	4.94	2.19
Copper	**0.75†**	0.70	2.46	0.72	0.56†	0.35	3.24	1.68
Range	2.9	4.1	5.7	2.9	17.3	1.9	3.2	3.1

Examples using survival endpoints:

Scenario	**Conclusion**
If a sediment is lethal to *H. azteca* only (bold)	Toxicity is likely due to nickel
If a sediment is lethal to *H. azteca* and *C. riparius* only (*)	Toxicity is likely due to cadmium
If a sediment is lethal to *H. azteca* and *Hexagenia* spp. only (†)	Toxicity is likely due to copper
If a sediment is lethal to *T. tubifex* only.	Toxicity is likely due to undetermined contaminant

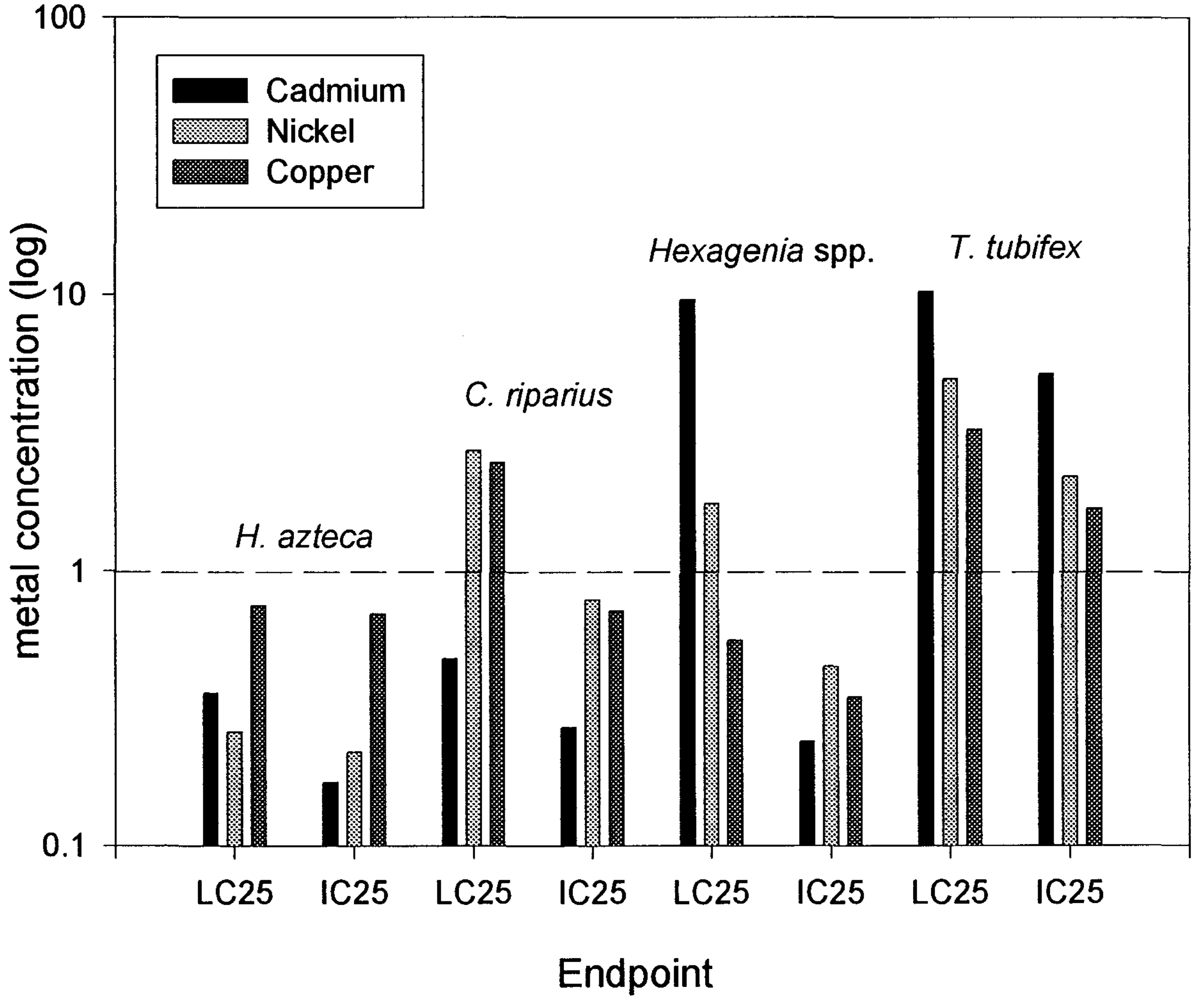

Figure 4.1 Bulk sediment LC25's and IC25's for each species divided by the geometric mean across all endpoints for each metal. Bars below the dotted line (< 1) indicate the more sensitive endpoints, bars above the dotted line indicate the less sensitive endpoints.

The above scenarios describe the diagnostic capabilities of the survival (LC25) endpoints. The chronic endpoints (IC25's) can be used to further confirm or check the cause of toxicity. For example, toxicity to *Hyalella*, *Chironomus* and *Hexagenia* growth will always be greater than to *Tubifex* reproduction if toxicity is attributed to cadmium, nickel or copper.

4.2 Field-Collected Sediment

4.2.1 Collingwood Harbour

Overall, species responses in sediments from Collingwood Harbour do not appear to reflect copper toxicity. From Table 4.2 (and Figure 4.1), survival and growth in *Hexagenia* are the most sensitive indicators of copper toxicity. Toxicity to *Hyalella* survival and growth and *Chironomus* growth may also occur. From Table 3.9, there is potential toxicity to *Hyalella* and *Chironomus* (survival) in Site C8, while sediment from Sites C6 and C8 showed potential toxicity to *Chironomus* (growth). These sites (C8 and C6) have the highest (525 μg/g) and second highest (357μg/g) copper concentration respectively (Table 3.8). Without knowing the species sensitivities to copper, one might speculate that copper is the plausible agent of (potential) toxicity in these sites. *Hexagenia* growth in the Collingwood Harbour sediments is quite high (Table 3.9), however, and growth exceeds the upper range in the non-toxic category in sites C6 & C8, which would not be expected with copper toxicity. Additionally, *Tubifex* is the least sensitive species to copper in the spiked sediment exposures (Table 4.2), yet toxicity to *Tubifex* reproduction is evident in sites C6, C7, C9, & C10. This further suggests that the causative agent of toxicity in the Collingwood Harbour sediments is not copper (or not copper alone). The high copper concentration in the sediments, which is well above the PEL for copper in three of the six sites (Table 3.8), is likely not bioavailable to the organisms. It is possible that another metal is responsible for toxicity to *Tubifex*, (there are elevated concentrations of zinc and lead above the PEL's in most sites), a combination of metals is responsible (additive or synergistic effects), or possibly that some unmeasured contaminant is causing the observed toxicity. Since the species sensitivities are not known for zinc or lead, or for metal mixtures, no strong conclusions can be drawn. The difference in *Tubifex's* response in the Collingwood Harbour sediments to that observed in the metal-spiked sediment exposures (*Tubifex* being the least sensitive organism to all metals tested) however, may also lead one to again question the validity of the present spiked sediment exposures for *Tubifex*, an organism that likely acquires metal primarily through sediment ingestion, not through the dissolved phase. This problem may be a limitation of the spiked metal exposures for *Tubifex*, and may need further resolution. The fact that the percent cocoon hatch and young production are affected in five of the six sites and not survival and cocoon production (Table 3.9) suggests that the mechanism of toxicity is in embryo formation inside the cocoon, or embryogenesis, similar results found by Reynoldson and Day (1998b) at these sites sampled previously.

Further studies with *Tubifex* would be useful to establish its sensitivity to other metals as well as other classes of compounds (organic contaminants). The data from Collingwood Harbour illustrate the importance of multiple single species testing, since the use of only one known sensitive species (i.e. *H. azteca*) may have lead to erroneous conclusions regarding the cause of sediment toxicity in this particular case.

From the multivariate analyses, it can be seen that the copper vector (as well as the Cd, Ni, and Fe vectors) travel orthogonal to the Collingwood Harbour sites, agreeing with above conclusions that copper is not the cause of toxicity in these sites. The grouping of the Collingwood Harbour sites (group 2) shows the similarity in Collingwood Harbour sites, with the exception of site C11 (group 3), which is the only site that did not show toxicity, or potential toxicity to *Tubifex*. Ordination reveals that TOC is important in modifying toxicity in Collingwood Harbour sites (but not at other sites) (Figure 3.3 & 3.4). Toxicity at the Collingwood Harbour sites may be attributable to an organic pollution problem. Since organic contaminant analyses were not performed on the Collingwood Harbour sediments, no strong conclusions can be drawn regarding the cause of toxicity at these sites. From Figure 3.3, there is no relationship between *Tubifex* survival and cocoon production (lower left corner) and percent cocoons hatched and young production (upper left corner), indicating that the mechanisms of toxicity are different in the Collingwood Harbour sites from other sites (i.e. sites De and S4).

4.2.2 Sudbury area

Species responses in the Sudbury area sediments appear to reflect nickel toxicity in site S2 only. From the spiked-sediment exposures, survival and growth in *Hyalella* are the most sensitive endpoints for nickel toxicity and reduced *Hexagenia* and *Chironomus* growth may also result (Table 4.2, Figure 4.1). Site S2, which has the second highest nickel concentration of the Sudbury sites (144 μg/g), above the PEL for nickel, is toxic to *Hyalella* (survival and growth), and is potentially toxic to *Hexagenia* (Table 3.9), supporting nickel toxicity.

Site S1 is toxic to *Hyalella* (survival and growth) only. While this may support nickel toxicity, the nickel concentration in this site is low (24 μg/g) (Table 3.8). Also, *Hexagenia* growth is highest in site S1 (Table 3.9), whereas a reduction in mayfly growth is likely with nickel toxicity (Table 4.2). It is possible that toxicity to *Hyalella* in site S1 may be related to physical structure of the sediment, i.e. the high sand content (94.4%) (Table 3.8). The high sand content of the sediment may also contribute to toxicity in site S2, which has the highest percent sand (95.3%) of the Sudbury sites.

Site S3 is toxic to *Hyalella* (survival) and potentially toxic to *Hyalella* (growth). There is no toxicity to the other three species. While this is possibly a response to nickel toxicity, as in site S1, the chemical data do not support nickel toxicity. S3 has a nickel concentration of 27μg/g and also a high percentage of sand particles (81.2%) (Table 3.8).

Sediment from Site S4 contains a mixture of metals present in high concentrations (copper 799 μg/g; nickel 342 μg/g; chromium 103 μg/g; cadmium 9.9 μg/g; (Table 3.8)), and a low pH was recorded in this site. Toxicity to *Hyalella* (zero survival and growth), *Hexagenia* (survival and growth), and *Chironomus* (growth) (Table 3.9) in site S4 supports copper toxicity, while toxicity to *Chironomus* (survival, along with *Hyalella* survival and growth and *Hexagenia* growth) supports cadmium toxicity (Table 4.2). Sediment from Site S4 is more lethal to *Tubifex* than *Chironomus*, however, which does not suggest cadmium or copper toxicity. Thus, toxicity in Site S4 may be due to a combination of several metals, to an unknown toxic agent, or to effects from the low pH (see below).

From the multivariate analysis, site S4 is associated with the metal vectors (especially the iron and copper vectors) suggesting that toxicity in this site is metal related. Sediments from Site S2, which supports nickel toxicity based on the species responses in this site compared to the sensitivities determined from the nickel-spiked exposures, is located orthogonal to the nickel vector (Figure 3.4). This indicates the possibility of another source of toxicity (i.e. physical nature of the sediment) as suggested above. Although particle size was not significant (at $p \leq 0.05$) based on the Monte-Carlo permutations, it may nonetheless be modifying test response. Sites S1and S3 are also orthogonal to the metal vectors, suggesting that toxicity in these sites is not metal related.

4.2.3 Rouyn-Noranda area

Species responses in the Rouyn-Noranda area sediments may reflect cadmium toxicity in Lakes Vaudray (Va), Joannès (Jo), and Default (De) but not in Lakes D'Alembert (Dl) or Opasatica (Op). Growth in *Hyalella*, *Hexagenia* and *Chironomus* are the most sensitive indicators of cadmium toxicity, and *Hyalella* and *Chironomus* survival may also be affected (Table 4.2, Figure 4.1). Sediment from Site Op shows toxicity to *Hyalella* (survival) only. It is unlikely that cadmium is responsible for this toxicity, as there is no growth reduction in *Hyalella*, *Hexagenia* or *Chironomus*, which are more sensitive indicators of cadmium toxicity. The cause of toxicity in sediment from Site Op is therefore undetermined, but should be noted that this site has the highest concentrations of chromium and nickel, which are both above the PEL's (Table 3.8).

It is possible that species responses in site Va and Jo are a result of cadmium toxicity, since sediment from both sites are toxic to *Hyalella* and *Chironomus* (survival). Additionally, sediment from Site Va is potentially toxic to *Hyalella* (growth). However, sediment cadmium concentrations from sites Va and Jo are below the PEL for cadmium (< 3.4 μg/g) (Table 3.8). Since the chemical data do not conclusively support cadmium toxicity (the exact sediment cadmium concentration in these sites is not known), it is difficult to conclude that cadmium is the causative agent of toxicity in these sediments.

While there is potential toxicity to *Hyalella* growth in site DI, this site is the least toxic to all four species, with no toxic responses in the other three species evident. The sediment cadmium concentration is below detection at this site (< 3.4 μg/g) (Table 3.8), therefore further evidence is needed to draw any strong conclusions regarding cadmium toxicity.

Site De contains a mixture of metals present in high concentrations (zinc 1728 μg/g; copper 1317 μg/g; lead 267 μg/g; cadmium 15.2 μg/g (Table 3.8)). Toxicity to *Hyalella* (zero survival and growth), *Hexagenia* (survival and growth), and *Chironomus* (growth) (Table 3.9), in this site supports copper toxicity (Table 4.2). however, the variability in *Hexagenia* survival is quite large in De (16.4), thus the data may also support cadmium toxicity. Site De is also toxic to *Tubifex* survival and reproduction (Table 3.9), while *Chironomus* survival is unaffected, suggesting the presence of other toxic agents.

From the multivariate analysis, the cadmium vector (as well as the Cu, Ni, and Fe vectors) travels orthogonal to all Rouyn-Noranda sites with the exception of site De (Figure 3.4). Site De is in the same proximity as the metal vectors (predominantly the cadmium vector) suggesting that toxicity in this site is metal related. Sites Va and Jo, however, which support cadmium toxicity, are located orthogonally to the cadmium vector, indicating that there may be other toxic agents present in these sites. The mechanism of *Tubifex* toxicity in sediment from site De (and site S4) is different from that observed in the Collingwood Harbour sediments with adult survival and cocoon formation affected in sites De and S4 (and not in Collingwood Harbour sediments) (Table 3.9). This is also seen in Figure 3.3, with *Tubifex* survival and cocoon production vectors (lower left corner) located opposite to site De (and S4) (upper right corner).

The direction of divergence of the field-collected sites from the reference sites may provide useful information on the identity of the toxicant(s). The field sites are located in different positions in ordination space relative to the reference sites, indicating that stressors acting on the species responses in the sites may be different. For instance, Collingwood Harbour sites C6, C7, C8, C10, Rouyn-Noranda site De, and Sudbury site S4 are located in different locations in Figure 3.5, suggesting that the stressors are different for these sites. The degree of divergence from the reference sites reveals the severity of impact (i.e. the further the distance from reference state in ordination space the more severe the toxicity). Sites S4 and De, which are located further from the reference sites than Collingwood Harbour sites C6, C7, C8, and C10, are overall the most toxic sites to all four species.

Potential Factors Affecting Toxicity in Field-Collected Sediments

Several factors may affect or contribute to the observed toxicity in the field-collected sediments including pH, particle size, total organic carbon, metal-metal interactions, and the sediment phases present.

The effects of pH on metal speciation and metal bioavailability and accumulation is complex and tends to be both metal and species specific (Wang 1987; Krantzberg and Stokes 1988; Mackie 1989; Schubauer-Berigan *et al.* 1993). The speciation of metals will determine the relative proportion and availability of the metal, and can also alter metal adsorption at cell surface membranes (Krantzberg and Stokes 1988; Marr *et al.* 1995). At the low pH recorded in Sudbury site S4 (ranging from 2.8 - 4.6 throughout the test period), a metal will tend to be in the more bioavailable free form (Marr *et al.* 1995; Taylor 1983), and the hydrogen ion itself may be toxic (Borgmann 1983).

The sediment constituents may also affect the distribution of the metal between sediment and water and hence metal availability to the organisms (Luoma 1989). Sediments with a high percentage of sand particles, (i.e. Sudbury sediments), have less surface area per unit mass than fine grained sediments, and thus have a lower number of surface binding sites per unit mass of the particle (Luoma 1989). Subsequently, more metal will be in solution in these sandy sediments. Site S4, which has the highest metal concentrations also has the highest percentage of clay and silt (33.7%). This may make the metals more available to sediment ingesting organisms, since it is generally thought that metals will be most associated with the clay and silt fractions of the sediment (Krumgalz 1989). Sandy sediments can also confound the results for physical reasons. For example, the mayfly generally shows reduced growth in the sandy sediments possibly due to abrasion of the particles, and *Hexagenia* generally require a finer textured sediment in which to build a burrow (Giesy *et al.* 1990). *Chironomus*, on the other hand grow well in sandy sediment. Ankley *et al.* (1994) found a positive relationship between the growth of *C. tentans* and the percentage of sand in sediment. Additionally, organisms may selectively feed or ingest certain particle sizes, thus this may also be an important factor in metal bioavailability (Campbell *et al.* 1988).

Interactive effects among metals may be an important factor influencing metal availability to organisms (Luoma 1983). The combination of several metals may lead to synergistic, additive or antagonistic effects depending on the metal species and metal concentrations present and the presence and concentration of ligands present (Luoma 1983; Wang 1987). This may be important in sites S4, De, C6, C7, & C8, which contain several metals in high concentrations, making it difficult to determine or distinguish between metal toxicity.

Toxicity test results can also be confounded by complexation with organic matter, and as such, the nature and amount of organic matter is important (Ankley *et al.* 1994; Lacey *et al.* 1999). Total organic carbon varied little between sites in each specific area, being fairly low in Rouyn-Noranda and Sudbury area sediments (0.1-0.2%), while in the Collingwood Harbour sediments it was ≥10-fold higher (Table 3.8). Thus complexation to organic carbon may have effectively reduced the concentration of the more bioavailable metal forms in the Collingwood Harbour sediments.

Finally, metals will partition between different solid phases of the sediment (Campbell *et al.* 1988; Luoma 1989), distributing mainly among iron and manganese oxide phases and organic material in oxic sediments, and precipitating as metal sulfides, or associating with iron sulfide in anoxic sediments (Luoma 1989). The chemical form of the metal is important since as certain forms are thought to be more available than others, and also, availability will vary depending on the metal (Wentzel *et al.* 1977b; Luoma 1983,1989). For instance, in examining copper bioaccumulation in tubificids (*L. hoffmeisteri* or *T. tubifex*), the amount of copper in the worm tissue was most correlated to the manganese oxide phase (easily reducible) of the sediment (more so than the water column) (Diks and Allen 1983; Campbell *et al.* 1988). Metals such as lead, however, were best correlated with the total metal concentration in the sediment (Campbell *et al.* 1988). The extraction procedure employed in this study is a soft acid (5% HCl) extraction, which is thought to extract the biologically available metal, but does not provide information of the quantity and quality of the solid phases of the sediment.

5. CONCLUSIONS

5.1 Spiked Water/Sediment Exposures

- In 96-hour water-only exposures, the species exhibit a wide range in sensitivity among the three metals, and this is most pronounced for the mayfly *Hexagenia* spp.
- In 96-hour water-only exposures, *H. azteca* is the most sensitive species to cadmium (*H. azteca* ≤ *C. riparius* < *T. tubifex* < *Hexagenia* spp. *H. azteca* and *C. riparius* are the most sensitive species to nickel (*H. azteca* = *C. riparius* < *T. tubifex* < *Hexagenia* spp.) and *C. riparius* is the most sensitive species to copper (*C. riparius* < *Hexagenia* spp. < *T. tubifex* ≤ *H. azteca*).
- The order in species sensitivity in the spiked sediment exposures follow that observed in the water-only exposures for cadmium, but not for nickel and copper.
- Survival in the spiked sediment exposures revealed that *H. azteca* is the most sensitive species to cadmium (*Hyalella* ≤ *Chironomus* << *Hexagenia* =*Tubifex*) and nickel (*Hyalella* < *Hexagenia* < *Chironomus* < *Tubifex*) and *Hexagenia* spp. and *H. azteca* are the most sensitive species to copper (*Hexagenia* = *Hyalella* < *Chironomus* < *Tubifex*).

- Reasons for observed differences in metal sensitivity between species and between exposure regimes (water-only vs., sediment exposure) may be due the following: exposure time, animal behaviour and metal uptake routes, test conditions, bioturbation as well as the chemical nature of the metal and the metal detoxification abilities of each species.
- While growth and reproduction are more sensitive indicators of toxicity than survival, they are not discriminatory endpoints due to the similar responses of the species to the three metals. For instance, *Hyalella* growth is always more affected than *Tubifex* reproduction and a growth reduction in *Chironomus* and *Hexagenia* occurs for all three metals. Due to the wide range in response of *Chironomus* and *Hexagenia* survival to the three metals, these two endpoints may be used to discriminate between cadmium, copper, and nickel toxicity in sediments. Three rules for distinguishing between cadmium, nickel, and copper toxicity are as follows. If a sediment is toxic to *Hyalella* and not to *Tubifex* and a growth reduction in *Chironomus* and *Hexagenia* occurs, then:

  1. Mortality to *Chironomus* ⟶ possibly cadmium toxicity
  2. Mortality to *Hexagenia* ⟶ possibly copper toxicity
  3. No mortality in *Chironomus* or *Hexagenia* ⟶ possibly nickel toxicity
- Survival and reproduction in *T. tubifex* are less sensitive to metals than survival and growth in the other three species. Spiked-sediment tests may not provide an appropriate means of determining metal sensitivity to an organism that likely acquires its metal from the solid phase (i.e. *Tubifex*).

- The differential responses to metal toxicity exhibited by the four species in the spiked exposures demonstrate the importance of multiple single species testing in evaluation of sediment toxicity.

5.2 Field-Collected Sediment

- Examination of the four species responses in Collingwood Harbour sediment revealed that copper is not likely the causative agent of toxicity. Thus, copper present in these sediments is likely not bioavailable to the organisms. While other metals in high concentrations (i.e. lead, zinc) are present in these sediments, species sensitivities to these metals and to metal mixtures are unknown, therefore the cause of toxicity in the Collingwood Harbour sediments remains undetermined.
- In the Sudbury sediments, species responses revealed several possible causes of toxicity. Sediment from site S4, which is the most toxic site to all four species, supports copper toxicity (*Hexagenia* mortality), cadmium toxicity (*Chironomus* mortality), and some other undetermined toxicant (*Tubifex* mortality), but not nickel toxicity. While species responses in sites S1 and S3 support nickel toxicity, it does not do so conclusively due to the low nickel concentrations in these sites. Toxicity to *Hyalella* and *Hexagenia* in site S2 support nickel toxicity. The presence of nuisance variables (i.e. a very high sand content in all sites and a low pH in site S4) may have also contributed to toxicity.

- In the Rouyn-Noranda sediments, species responses revealed cadmium as the likely cause of toxicity in certain sites. Toxicity in sediments from sites Va and Jo are consistent with cadmium toxicity (*Hyalella* and *Chironomus* mortality). However, toxicity to *Tubifex* (and not *Chironomus*) in sediment from site De provides evidence of another undetermined toxicant present, while combined *Hyalella* and *Hexagenia* mortality may reflect copper toxicity.
- Multivariate analyses revealed that the three metals of interest, cadmium, copper, and nickel, as well as aluminium, iron, magnesium, manganese, and total organic carbon are significant measured environmental attributes in the field-collected sediments. Relationships between these specific environmental attributes and the species responses likely exist determined by the location of the vectors in ordination space and by multiple regression.
- Multivariate analyses allowed the graphical presentation of the field-collected sediments demonstrating the relationships between the field sites.
- The use of multivariate methods is a useful tool in assessing sediment toxicity relative to a group of reference sites. The probability ellipses (around the reference sites) appear to provide a sensitive measure of the degree of sediment toxicity, revealing nine of the fifteen sites severely toxic (C6, C7, C8, C10, Va, De, S2, S3, S4); four sites toxic (C9, Op, Jo, S1); one site potentially toxic (C11), and one site non-toxic (DI). In the event of multiple stressors, these multivariate methods may also be implemented diagnostically to suggest the primary source of toxicity.

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Appendix A

Analytical Results from Water-only and Spiked Sediment Exposures

Table A.1 Analytical results from cadmium-spiked water-only exposures.

Species/Test	Nominal mg/L	Measured mg/L	Species/Test	Nominal mg/L	Measured mg/L
Hyalella/1	0	0.000	*Hexagenia/1*	0	0.000
	0.001	0.001		0.5	0.585
	0.0025	0.003		1	1.114
	0.005	0.006		5	5.574
	0.01	0.011		10	10.92
	0.025	0.030		50	56.13
	0.05	0.058		100	106.1
Hyalella/2	0	0.000	*Hexagenia/2*	0	0.226
	0.001	0.001		0.5	0.4982
	0.0025	0.003		1	1.049
	0.005	0.006		5	5.261
	0.01	0.011		10	10.24
	0.025	0.038		25	26.15
	0.05	0.054		50	52.28
Hyalella/3	0	0.000	*Hexagenia/3*	0	<0.034
	0.001	0.001		0.25	0.2696
	0.0025	0.003		0.5	0.5336
	0.005	0.005		1	1.092
	0.01	0.013		5	5.469
	0.025	0.028		10	11.22
	0.05	0.092		50	56.37
Chironomus/1	0	0.000	*Tubifex/1*	0	<0.034
	0.001	0.001		0.1	0.1051
	0.0025	0.003		0.5	0.5276
	0.005	0.005		0.75	0.7935
	0.01	*		1	1.068
	0.025	0.028		2.5	2.711
	0.05	0.0485		5	5.314
Chironomus/2	0	0.000	*Tubifex/2*	0	<0.034
	0.001	0.001		0.1	0.1187
	0.0025	0.003		0.5	0.5772
	0.005	0.006		0.75	0.8763
	0.01	0.012		1	1.201
	0.025	0.028		2.5	2.904
	0.05	0.0589		5	5.901
Chironomus/3	0	0.000	*Tubifex/3*	0	<0.034
	0.001	0.001		0.1	0.1215
	0.0025	0.003		0.5	0.5781
	0.005	0.006		0.75	0.8443
	0.01	0.011		1	1.131
	0.025	0.028		2.5	2.791
	0.05	0.0521		5	5.588

** = sample lost*

Table A.2 Analytical results from nickel-spiked water-only exposures.

Species/Test	Nominal mg/L	Measured mg/L
Hyalella/1	0	0.3254
	0.5	0.5917
	1	1.096
	5	5.315
	10	10.51
	25	26.46
	50	*
Hyalella/2	0	0.0572
	0.5	0.5188
	1	1.057
	5	5.116
	10	10.65
	25	26.41
	50	52.34
Hyalella/3	0	0.0242
	0.5	0.6007
	1	1.058
	5	5.234
	10	10.56
	25	26.23
	50	52.15
Hyalella/4	0	0.0242
	0.5	0.6007
	1	1.058
	5	5.234
	10	10.56
	25	26.23
	50	52.15
Chironomus/1	0	0.000
	0.1	0.1253
	0.5	0.5446
	1	1.027
	5	5.332
	10	10.46
	50	52.91
Chironomus/2	0	0.000
	0.1	0.0951
	0.5	0.5548
	1	1.143
	5	5.535
	10	11.41
	50	54.43
Chironomus/3	0	<0.02
	0.1	0.095
	0.5	0.5068
	1	1.054
	5	5.191
	10	10.44
	50	52.16
Chironomus/4	0 ppm	0.4371
	0.1	0.1651
	0.5	0.5848
	1	1.145
	5	5.762
	10	11.41
	50	55.25
Hexagenia/1	0	0.000
	0.5	0.511
	1	0.951
	5	5.052
	10	9.987
	50	52.17
	100	105.9
Hexagenia/2	0	0.000
	1	1.238
	5	5.167
	10	10.43
	50	51.39
	75	78.61
	100	104.2
Hexagenia/3	0	0.1455
	1	1.071
	5	5.356
	10	10.52
	50	51.38
	75	78.39
	100	109.3
Tubifex/1	0	0.000
	5	5.091
	10	10.271
	25	27.26
	50	50.97
	100	98.01
Tubifex/2	0	<0.02
	5	4.917
	10	9.814
	25	26.98
	50	53.06
	100	109.6
Tubifex/3	0	0.0538
	5	5.001
	10	10.48
	25	27.54
	50	53.48
	100	113.8

** = sample lost*

Table A.3 Analytical results from copper-spiked water-only exposures.

Species/Test	Nominal mg/L	Measured mg/L	Species/Test	Nominal mg/L	Measured mg/L
Hyalella/1	0	0.0025	*Hexagenia/1*	0	0.0023
	0.025	0.0251		0.01	0.0108
	0.05	0.0507		0.025	0.031
	0.1	0.1386		0.05	0.049
	0.25	0.1914		0.1	0.099
	0.5	0.4728		0.5	0.497
	1	<0.01		1	1.019
Hyalella/2	0	0.0023	*Hexagenia/2*	0	0.0033
	0.025	0.0267		0.01	0.0111
	0.05	*		0.025	0.023
	0.1	0.1017		0.05	0.051
	0.25	0.1968		0.1	0.104
	0.5	0.4435		0.5	0.498
	1	<0.01		1	1.01
Hyalella/3	0	0.0016	*Hexagenia/3*	0	<0.01
	0.025	0.0255		0.01	0.0126
	0.05	0.0477		0.025	0.0224
	0.1	0.0988		0.05	0.0488
	0.25	0.2626		0.1	0.1017
	0.5	0.522		0.5	0.4617
	1	0.9919		1	0.8941
Chironomus/1	0	0.0147	*Tubifex/1*	0	0.0012
	0.025	0.0276		0.05	0.0437
	0.05	0.0529		0.1	0.0566
	0.1	0.0974		0.25	0.1706
	0.5	0.4923		0.5	0.4081
	1	0.9738		1	0.9392
	5	5.099		5	4.751
Chironomus/2	0	<0.01	*Tubifex/2*	0	<0.01
	0.025	0.0236		0.05	0.0478
	0.05	0.0468		0.1	0.0965
	0.1	0.1003		0.25	0.2471
	0.5	0.5113		0.5	0.4613
	1	1.032		1	0.9886
	5	5.301		5	5.071
Chironomus/3	0	<0.01	*Tubifex/3*	0	<0.01
	0.025	0.0231		0.05	0.0466
	0.05	0.0467		0.1	0.0976
	0.1	0.1002		0.25	0.2623
	0.5	0.5151		0.5	0.4668
	1	1.006		1	0.9887
	5	5.117		2.5	2.441

** = sample lost*

Table A.4. Analytical results from cadmium-spiked sediment exposures for *Hyalella* and *Chironomus*.

Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*	Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*
Hyalella/1	0	0.001	0.001	0.8001		*Chironomus/1*	0	0.001	0.001	0.8001	
	10	0.001	0.002	9.641	96.41		10	0.001	0.002	9.641	96.41
	20	0.002	0.004	16.33	81.65		20	0.002	0.004	16.33	81.65
	41	*	*	*	*		41	*	*	*	*
	81	0.005	0.016	64.73	79.91		81	0.005	0.016	64.73	79.91
	122	0.027	0.037	100.3	82.21		122	0.027	0.037	100.3	82.21
	163	*	*	*	*		163	*	*	*	*
	203	0.098	0.171	155.7	76.70		203	0.098	0.171	155.7	76.70
Hyalella/2	0	0.000	0.000	0.34		*Chironomus/2*	0	0.000	0.000	0.34	
	6	0.001	0.001	4.601	76.68		6	0.000	0.001	5.238	87.30
	11	0.001	0.004	9.281	84.37		11	0.000	0.003	9.117	82.88
	20	0.002	0.004	13.89	69.45		20	0.001	0.007	15.98	79.90
	37	0.004	0.009	28.81	77.86		37	0.002	0.012	28.08	75.89
	65	0.004	0.036	48.54	74.68		65	0.004	0.036	50.66	77.94
	114	0.015	0.048	89.25	78.29		114	*	0.065	92.08	80.77
	203	0.031	0.085	144.2	71.03		203	0.007	0.115	143.5	70.69
Hyalella/3	0	0.000	0.000	0.34		*Chironomus/3*	0	0.000	0.000	0.34	
	6	0.000	0.001	5.238	87.30		6	0.001	0.002	5.086	84.77
	11	0.000	0.003	9.117	82.88		11	0.001	0.003	9.518	86.53
	20	0.001	0.007	16.31	81.55		20	0.002	0.005	17.54	87.70
	37	0.002	0.012	28.08	75.89		37	0.003	0.02	27.61	74.62
	65	0.004	0.036	50.66	77.94		65	0.003	0.033	47.08	72.43
	114	*	0.065	92.08	80.77		114	0.018	0.052	95.19	83.50
	203	0.007	0.115	143.5	70.69		203	0.03	0.101	167.1	82.32

** = sample lost*

Table A.6. Analytical results from nickel-spiked sediment exposures for *Hyalella* and *Chironomus*.

Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*
Hyalella/1	0	<0.02	4.099	0.6448	
	23	0.0211	1.211	20.78	90.35
	40	0.0281	1.742	30.78	76.95
	73	0.0974	0.7671	51.91	71.11
	227	0.4109	1.354	184.6	81.32
	405	1.162	2.538	374.2	92.40
Hyalella/2	0	<.02	<.02	6.197	
	23	<.02	0.024	28.67	124.65
	40	0.051	0.089	44.48	111.20
	73	0.142	0.221	89.57	122.70
	227	0.2991	1.284	174.8	77.00
	405	0.5262	1.981	239.3	59.09
Hyalella/3	0	0.4482	<.02	8.539	
	23	0.0336	0.0298	22.21	96.57
	40	0.0326	0.0751	34.95	87.38
	73	0.0662	0.1525	43.45	59.52
	227	0.2435	0.7603	147.3	64.89
	405	0.4691	1.231	219.8	54.27
Hyalella/4	0	0.3053	0.0233	7.078	
	23	0.2312	0.0667	18.93	82.30
	40	0.0851	0.1456	33.91	84.78
	73	0.0696	0.2796	50.91	69.74
	227	0.492	1.505	125.5	55.29
	405	1.092	4.006	261.8	64.64

Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*
Chironomus/1	0	<.02	<.02	6.197	
	23	<.02	0.024	28.67	124.65
	40	0.051	0.089	44.48	111.20
	73	0.142	0.221	89.57	122.70
	227	0.2991	1.284	174.8	77.00
	405	0.5262	1.981	239.3	59.09
	728	0.9626	3.619	459.9	63.17
	2266	130.6	107.5	1279	56.44
Chironomus/2	0	0.4482	<.02	8.539	
	23	0.0336	0.0298	22.21	96.57
	40	0.0326	0.0751	34.95	87.38
	73	0.0662	0.1525	43.45	59.52
	227	0.2435	0.7603	147.3	64.89
	405	0.4691	1.231	219.8	54.27
	728	1.311	3.535	364.2	50.03
	2266	113.7	104.5	1089	48.06
Chironomus/3	0	0.3053	0.0233	7.078	
	23	0.2312	0.0667	18.93	82.30
	40	0.0851	0.1456	33.91	84.78
	73	0.0696	0.2796	50.91	69.74
	227	0.492	1.505	125.5	55.29
	405	1.092	4.006	261.8	64.64
	728	1.236	5.173	442.8	60.82
	2266	113.3	*	1091	48.15

** = sample lost*

Table A.7. Analytical results from nickel-spiked sediment exposures for *Hexagenia* and *Tubifex*.

Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*
Hexagenia/1	0	<0.2	5.023	1.048	
	40	0.045	0.4149	29.34	73.35
	130	0.091	0.4138	99.71	76.70
	405	0.6954	1.827	314.3	77.60
	1295	7.779	20.89	887.9	68.56
	4047	490.2	478	2144	52.98
Hexagenia/2	0	<0.02	0.878	2.955	
	40	0.045	0.423	25.94	64.85
	130	0.284	1.057	107.1	82.38
	405	1.583	3.128	259.7	64.12
	1296	37.81	38.92	711.7	54.92
	4050	533.3	531.3	1564	38.62
Hexagenia/3	0	0.155	0.227	5.213	
	40	<0.02	0.406	22.54	56.35
	130	0.134	0.756	85.09	65.45
	405	1.641	2.716	229.1	56.57
	1296	22.93	28.43	646.9	49.92
	4050	386.1	327.4	1565	38.64

Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*
Tubifex/1	0	<0.2	5.023	1.048	
	40	0.045	0.4149	29.34	73.35
	130	0.091	0.4138	99.71	76.70
	405	0.6954	1.827	314.3	77.60
	1295	7.779	20.89	887.9	68.56
	4047	490.2	478	2144	52.98
Tubifex/2	0	<0.02	0.878	2.955	
	40	0.045	0.423	25.94	64.85
	130	0.284	1.057	107.1	82.38
	405	1.583	3.128	259.7	64.12
	1296	37.81	38.92	711.7	54.92
	4050	533.3	531.3	1564	38.62
Tubifex/3	0	0.155	0.227	5.213	
	40	<0.02	0.406	22.54	56.35
	130	0.134	0.756	85.09	65.45
	405	1.641	2.716	229.1	56.57
	1296	22.93	28.43	646.9	49.92
	4050	386.1	327.4	1565	38.64

Table A.8. Analytical results from copper-spiked sediment exposures for *Hyalella* and *Chironomus*.

Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*
Hyalella/1	0	0.00325	0.00559	12.68	
	15	0.00485	0.01411	30.31	202.07
	27	0.00602	0.0199	39.08	144.74
	48	0.00949	0.03238	59.32	123.58
	150	0.0302	0.0588	130.2	86.80
	268	0.0327	0.1085	243.4	90.82
Hyalella/2	0	0.00292	0.00789	12.96	
	15	0.0047	0.01377	23.47	156.47
	27	0.00728	0.01825	43.52	161.19
	48	0.00754	0.0251	66.88	139.33
	150	0.0134	0.0763	142.8	95.20
	268	0.0263	0.1151	282.6	105.45
Hyalella/3	0	0.00365	0.00439	12.72	
	15	0.00514	0.00979	27.85	185.67
	27	0.00535	0.0124	30.27	112.11
	48	0.0076	0.0135	52.58	109.54
	150	0.0332	0.0542	155.1	103.40
	268	0.0983	0.1116	250.5	93.47
Chironomus/1	0	0.00325	0.00559	12.68	
	15	0.00485	0.01411	30.31	202.07
	27	0.00602	0.0199	39.08	144.74
	48	0.00949	0.03238	59.32	123.58
	150	0.0302	0.0588	130.2	86.80
	268	0.0327	0.1085	243.4	90.82
	483	0.0512	0.3084	563.2	116.60
	1502	0.2561	1.071	1368	91.08
Chironomus/2	0	0.00292	0.00789	12.96	
	15	0.0047	0.01377	23.47	156.47
	27	0.00728	0.01825	43.52	161.19
	48	0.00754	0.0251	66.88	139.33
	150	0.0134	0.0763	142.8	95.20
	268	0.0263	0.1151	282.6	105.45
	483	0.1313	0.2108	451.9	93.56
	1502	0.2135	0.7861	1408	93.74
Chrionomus/3	0	0.00365	0.00439	12.72	
	15	0.00514	0.00979	27.85	185.67
	27	0.00535	0.0124	30.27	112.11
	48	0.0076	0.0135	52.58	109.54
	150	0.0332	0.0542	155.1	103.40
	268	0.0521	0.1116	250.5	93.47
	483	0.0983	0.1786	561.3	116.21
	1502	0.1709	0.6265	1433	95.41

Table A.9. Analytical results from copper-spiked sediment exposures for *Hexagenia* and *Tubifex*.

Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*	Species/Test	Nominal Conc *mg/kg*	Overlying Water *mg/L*	Pore Water *mg/L*	Bulk Sediment *mg/kg*	Spike Verification *%*
Hexagenia/1	0	0.00637	0.00633	11.65		*Tubifex/1*	0	0.00637	0.00633	11.65	
	13	0.0098	0.00793	23.44	180.31		13	0.0098	0.00793	23.44	180.31
	27	0.01945	0.01735	39.15	145.00		27	0.01945	0.01735	39.15	145.00
	134	0.0457	0.0886	127.8	95.37		134	0.0457	0.0886	127.8	95.37
	268	0.0613	0.5189	237.8	88.73		268	0.0613	0.5189	237.8	88.73
	1342	0.2097	1.862	1211	90.24		1342	0.2097	1.862	1211	90.24
Hexagenia/2	0	0.00305	0.00779	14.19		*Tubifex/2*	0	0.00305	0.00779	14.19	
	13	0.00247	0.02043	29.21	224.69		13	0.00247	0.02043	29.21	224.69
	27	0.00265	0.02152	33.94	125.70		27	0.00265	0.02152	33.94	125.70
	134	0.03672	0.0823	129.9	96.94		134	0.03672	0.0823	129.9	96.94
	268	0.0435	0.1806	246.9	92.13		268	0.0435	0.1806	246.9	92.13
	1342	0.0847	0.5703	1142	85.10		1342	0.0847	0.5703	1142	85.10
Hexagenia/3	0	0.00412	0.0062	6.423		*Tubifex/3*	0	0.00412	0.0062	6.423	
	13	0.00646	0.0149	33.89	260.69		13	0.00646	0.0149	33.89	260.69
	27	0.02886	0.0227	44.91	166.33		27	0.02886	0.0227	44.91	166.33
	134	0.0187	0.1012	146.1	109.03		134	0.0187	0.1012	146.1	109.03
	268	0.0316	0.5737	270.5	100.93		268	0.0316	0.5737	270.5	100.93
	1342	0.1776	1.862	1189	88.60		1342	0.1776	1.862	1189	88.60

Appendix B

Raw Data for Spiked Sediment Tests

CADMIUM SPIKES

H. azteca - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	86.67	88.89	3.85	4.33	0.315	0.39	0.07	16.87
	2								
	3	93.33				0.438			
	4	86.67				0.417			
10	1		82.22	10.18	12.39		0.27	0.04	14.75
	2	93.33				0.321			
	3	80				0.253			
	4	73.33				0.249			
41	1	60	73.33	9.43	12.86	0.12	0.14	0.04	28.83
	2	80				0.158			
	3	73.33				0.175			
	4	80				0.088			
81	1	33.33	38.33	6.39	16.66	0.112	0.10	0.03	26.95
	2	40				0.117			
	3	46.67				0.113			
	4	33.33				0.06			
122	1	0	1.67	3.34	200.00		0.34		
	2	6.67				0.34			
	3	0							
	4	0							
163	1	0	0.00	0.00					
	2	0							
	3	0							
	4	0							
203	1	0	0.00	0.00					
	2	0							
	3	0							
	4	0							

H. azteca - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	96.67	6.66	6.89	0.332	0.34	0.06	16.36
	2	86.67				0.295			
	3	100				0.419			
	4	100				0.31			
10	1	93.33	93.33	9.43	10.10	0.299	0.27	0.03	9.78
	2	80				0.28			
	3	100				0.236			
	4	100				0.281			
20	1	73.33	86.67	10.89	12.56	0.215	0.22	0.02	7.46
	2	100				0.222			
	3	86.67				0.232			
	4	86.67				0.194			
41	1	86.67	78.33	19.91	25.42	0.192	0.18	0.02	9.27
	2	100				0.172			
	3	73.33				0.16			
	4	53.33				0.195			
81	1	40	48.33	6.38	13.20	0.118	0.11	0.01	12.03
	2	53.33				0.114			
	3	46.67				0.089			
	4	53.33				0.106			
122	1	46.67	26.67	14.40	54.01	0.093	0.09	0.04	41.15
	2	20				0.083			
	3	26.67				0.04			
	4	13.33				0.125			
163	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
	3	0				-			
	4	0				-			

H. azteca - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	93.33	93.33	0.00	0.00	0.291	0.35	0.08	22.14
	2	93.33				0.399			
10	1	93.33	93.33	0.00	0.00	0.231	0.30	0.10	33.78
	2	93.33				0.376			
20	1	80	73.34	9.43	12.85	0.363	0.29	0.11	38.70
	2	66.67				0.207			
41	1	93.33	93.33	0.00	0.00	0.249	0.28	0.04	14.07
	2	93.33				0.304			
81	1	80	80.00	0.00	0.00	0.108	0.12	0.01	12.53
	2	80				0.129			
122	1	20	33.34	18.86	56.57	0.103	0.08	0.03	30.96
	2	46.67				0.066			
163	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
203	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

CADMIUM SPIKES

C. riparius - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	86.67	85.00	3.33	3.92	0.343	0.399	0.04	10.46
	2	80				0.436			
	3	86.67				0.425			
	4	86.67				0.392			
20	1	73.33	80.00	12.17	15.21	0.405	0.395	0.05	12.10
	2	93.33				0.325			
	3	86.67				0.425			
	4	66.67				0.426			
41	1	80	86.67	5.44	6.28	0.284	0.269	0.02	7.11
	2	86.67				0.254			
	3	86.67				0.287			
	4	93.33				0.251			
81	1	53.33	60.00	9.43	15.71	0.119	0.143	0.02	13.45
	2	53.33				0.16			
	3	60				0.157			
	4	73.33				0.136			
122	1	33.33	30.00	8.61	28.69	0.0124	0.005	0.01	118.97
	2	40				0.0087			
	3	26.67				0			
	4	20				0			
163	1	0	3.34	3.85	115.47		0.000	0.00	
	2	0							
	3	6.67				0			
	4	6.67				0			
203	1	0	0.00	0.00					
	2	0							
	3	0							
	4	0							

C. riparius - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	86.67	86.67	0.00	0.00	0.358	0.34	0.02	7.05
	2	86.67				0.324			
10	1	60	73.34	18.86	25.72	0.402	0.35	0.08	21.94
	2	86.67				0.294			
20	1	86.67	80.00	9.43	11.79	0.278	0.30	0.04	12.31
	2	73.33				0.331			
41	1	93.33	83.33	14.14	16.97	0.194	0.24	0.07	28.28
	2	73.33				0.291			
81	1	73.33	63.33	14.14	22.33	0.131	0.10	0.05	53.59
	2	53.33				0.059			
122	1	6.67	16.67	14.14	84.84	0	0.02	0.03	141.42
	2	26.67				0.048			
163	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
203	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

C. riparius - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	0.328	0.33	0.01	2.12
	2	100				0.338			
10	1	100	93.34	9.43	10.10	0.395	0.36	0.04	12.26
	2	86.67				0.332			
20	1	100	100.00	0.00	0.00	0.295	0.32	0.03	9.40
	2	100				0.337			
41	1	100	100.00	0.00	0.00	0.294	0.28	0.02	7.87
	2	100				0.263			
81	1	86.67	86.67	0.00	0.00	0.168	0.20	0.04	21.12
	2	86.67				0.227			
122	1	33.33	16.67	23.57	141.42	0.052	0.05		
	2	0							
163	1	6.67	6.67	0.00	-	0.15	0.08	0.11	141.42
	2	6.67				0			
203	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

CADMIUM SPIKES

Hexagenia spp. - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	3.691	3.67	0.03	0.91
	2	100				3.695			
	3	100				3.635			
20	1	100	100.00	0.00	0.00	0.663	1.01	0.49	48.48
	2	100				1.571			
	3	100				0.798			
65	1	100	100.00	0.00	0.00	0.526	0.37	0.15	41.10
	2	100				0.225			
	3	100				0.352			
203	1	100	93.33	5.77	6.19	0.075	0.05	0.12	227.29
	2	90				0.157			
	3	90				-0.076			
650	1	80	86.67	11.55	13.32	-0.45	-0.30	0.14	-47.15
	2	80				-0.169			
	3	100				-0.281			
2032	1	0	0.00	0.00					
	2	0							
	3	0							

Hexagenia spp. - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	90	95.00	5.77	6.08	4.003	3.73	0.23	6.14
	2	100				3.728			
	3	90				3.443			
	4	100				3.756			
20	1	100	100.00	0.00	0.00	1.398	1.35	0.49	36.58
	2	100				0.836			
	3					1.822			
	4								
65	1	90	97.50	5.00	5.13	0.566	0.61	0.21	33.46
	2	100				0.689			
	3	100				0.358			
	4	100				0.845			
203	1	90	97.50	5.00	5.13		0.06	0.01	19.98
	2	100				0.05			
	3	100				0.072			
	4	100				0.054			
650	1	70	70.00	0.00	0.00	-0.148	-0.16	0.03	-18.08
	2	70				-0.192			
	3	70				-0.127			
	4	70				-0.177			
2032	1	0	0.00	0.00					
	2	0							
	3	0							
	4	0							

Hexagenia spp. - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	4.355	4.21	0.21	4.99
	2	100				4.058			
50	1	100	95.00	7.07	7.44	0.41	0.48	0.09	19.35
	2	90				0.54			
100	1	100	100.00	-	-	0.271	0.27	-	-
150	1	90	90.00	-	-	0.208	0.21	-	-
200	1	100	100.00	-	-	0.017	0.02	-	-
250	1	100	100.00	-	-	0.12	0.12	-	-
300	1	100	100.00	-	-	0.098	0.10	-	-
500	1	70	70.00	-	-	-0.098	-0.10	-	-
1000	1	30	30.00	-	-	-0.335	-0.34	-	-

CADMIUM SPIKES

T. tubifex - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100	0	0	39	9.75	9.083	0.801	8.819	22	56.41	59.29	4.07	6.86	67.00	16.75	21.38	6.54	30.60
	2	100				37	9.25				23	62.16				104	26.00			
	3	100				33	8.25				15	45.45				85	21.25			
	4	100				40	10.00				24	60.00				84	21.00			
	5	100				37	9.25				20	54.05				102	25.50			
	6	100				32	8.00				20	62.50				96	24.00			
113	1	100	100	0	0	39	9.75	9.000	0.894	9.938	23	58.97	61.92	4.17	6.73	91.00	22.75	23.38	0.88	3.78
	2	100				37	9.25				24	64.86				96	24.00			
	3	100				38	9.50				26	68.42				112	28.00			
	4	100				36	9.00				24	66.67				93	23.25			
	5	100				37	9.25				24	64.86				106	26.50			
	6	100				29	7.25				23	79.31				95	23.75			
227	1	100	100	0	0	41	10.25	9.083	1.357	14.940	27	65.85	59.71	8.68	14.54	105.00	26.25	23.13	4.42	19.11
	2	100				28	7.00				15	53.57				80	20.00			
	3	100				33	8.25				19	57.58				93	23.25			
	4	100				37	9.25				24	64.86				100	25.00			
	5	100				43	10.75				27	62.79				108	27.00			
	6	100				36	9.00				25	69.44				97	24.25			
340	1	100	100	0	0	33	8.25	8.500	0.742	8.725	23	69.70	66.79	4.11	6.15	91.00	22.75	24.38	2.30	9.43
	2	100				36	9.00				23	63.89				104	26.00			
	3	100				33	8.25				22	66.67				100	25.00			
	4	100				37	9.25				24	64.86				122	30.50			
	5	100				36	9.00				20	55.56				96	24.00			
	6	100				29	7.25				23	79.31				67	16.75			
454	1	100	100	0	0	39	9.75	7.458	1.444	19.362	20	51.28	53.90	3.71	6.87	91.00	22.75	21.38	1.94	9.10
	2	100				23	5.75				13	56.52				80	20.00			
	3	100				34	8.50				21	61.76				83	20.75			
	4	100				26	6.50				18	69.23				65	16.25			
	5	100				28	7.00				20	71.43				50	12.50			
	6	100				29	7.25				19	65.52				61	15.25			
567	1	100	100	0	0	26	6.50	6.917	1.730	25.007	17	65.38	3.21	4.91	152.94	32	8.00	15.25	190.63	1250.00
	2	100				33	8.25				19	57.58				63	15.75			
	3	100				28	7.00				23	82.14				58	14.50			
	4	100				17	4.25				15	88.24				56	14.00			
	5	100				37	9.25				20	54.05				57	14.25			
	6	100				25	6.25				23	92.00				27	6.75			
681	1	100	100	0	0	30	7.50	6.725	0.868	12.905	24	80.00	7.69	9.62	125.00	22	5.50	17.12	311.33	1818.18
	2	100				26	6.50				18	69.23				40	10.00			
	3	100																		
	4	100				22	5.50				19	86.36				36	9.00			
	5	100				61	7.63				34	55.74				68	8.50			
	6	100				26	6.50				14	53.85				33	8.25			
1134	1	75	29.1667	29.226	100.2	2	0.57	1.006	0.373	37.035	2	100.00	1.03	1.03	100.00	3	0.86	2.00	233.33	11666.67
	2	25				3	1.20				3	100.00				7	2.80			
	3	50				2	0.67				1	50.00				5	1.67			
	4	0				2	1.00				2	100.00				2	1.00			
	5	0				2	1.00				2	100.00				5	2.50			
	6	25				4	1.60				4	100.00				2	0.80			
2033	1	0	0	0		0	0.00	0.000	0.000		0	-	-	-	-	0	0.00	-	-	-
	2	0				0	0.00				0	-				0	0.00			
	3	0				0	0.00				0	-				0	0.00			
	4	0				0	0.00				0	-				0	0.00			
	5	0				0	0.00				0	-				0	0.00			
	6	0				0	0.00				0	-				0	0.00			

CADMIUM SPIKES

T. tubifex - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100	0	0	42	10.50	9.00	1.43	15.88	26	61.90	59.00	8.16	13.84	51	12.75	16.88	5.84	34.61
	2	100				29	7.25				19	65.52				52	13.00			
	3	100				34	8.50				16	47.06				101	25.25			
	4	100				39	9.75				24	61.54				66	16.50			
20	1	100	100	0	0	32	8.00	9.38	1.38	14.69	19	59.38	63.75	2.96	4.64	79	19.75	19.63	3.26	16.59
	2	100				40	10.00				26	65.00				74	18.50			
	3	100				34	8.50				22	64.71				65	16.25			
	4	100				44	11.00				29	65.91				96	24.00			
65	1	100	100	0	0	34	8.50	9.19	0.52	5.61	20	58.82	61.81	3.11	5.03	54	13.50	19.50	5.15	26.40
	2	100				37	9.25				24	64.86				80	20.00			
	3	100				39	9.75				25	64.10				104	26.00			
	4	100				37	9.25				22	59.46				74	18.50			
203	1	100	100	0	0	37	9.25	9.06	1.09	12.00	25	67.57	64.31	6.94	10.80	91	22.75	20.44	4.44	21.73
	2	100				42	10.50				26	61.90				61	15.25			
	3	100				32	8.00				23	71.88				74	18.50			
	4	100				34	8.50				19	55.88				101	25.25			
650	1	100	93.75	12.5	13.33	29	7.25	7.76	0.74	9.59	13	44.83	53.32	8.65	16.22	31	7.75	7.70	2.27	29.52
	2	100				34	8.50				19	55.88				32	8.00			
	3	100				28	7.00				18	64.29				19	4.75			
	4	75				29	8.29				14	48.28				36	10.29			
2032	1	0	0	0		0	0.00	0.00	0.00		0	0	-	-	-	0	0.00	0.00	0.00	-
	2	0				0	0.00				0	0				0	0.00			
	3	0				0	0.00				0	0				0	0.00			
	4	0				0	0.00				0	0				0	0.00			

T. tubifex - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100	0	0	41	10.25	9.75	0.79	8.11	23	56.10	61.59	5.37	8.72	88	22.00	21.13	2.65	12.54
	2	100				35	8.75				23	65.71				69	17.25			
	3	100				42	10.50				28	66.67				93	23.25			
	4	100				38	9.50				22	57.89				88	22.00			
20	1	100	100	0	0	36	9.00	9.44	0.72	7.61	24	66.67	60.93	5.20	8.54	92	23.00	22.50	0.98	4.35
	2	100				42	10.50				26	61.90				94	23.50			
	3	100				36	9.00				22	61.11				85	21.25			
	4	100				37	9.25				20	54.05				89	22.25			
65	1	100	100	0	0	41	10.25	9.50	0.79	8.32	22	53.66	55.21	5.10	9.23	73	18.25	18.56	2.93	15.76
	2	100				40	10.00				22	55.00				59	14.75			
	3	100				34	8.50				17	50.00				87	21.75			
	4	100				37	9.25				23	62.16				78	19.50			
203	1	100	93.75	12.5	13.33	39	9.75	10.42	0.82	7.88	26	66.67	62.27	4.63	7.44	117	29.25	24.46	4.86	19.88
	2	100				39	9.75				25	64.10				72	18.00			
	3	100				43	10.75				24	55.81				95	23.75			
	4	75				40	11.43				25	62.50				94	26.86			
650	1	100	87.5	25		25	6.25	5.33	1.22	22.86	21	84.00	85.93	6.25	7.28	13	3.25	2.65	0.97	36.49
	2	100				15	3.75				12	80.00				5	1.25			
	3	100				20	5.00				17	85.00				11	2.75			
	4	50				19	6.33				18	94.74				10	3.33			
2032	1	0	0	0		0	0.00	0.00	0.00		0	0	-	-	-	0	0.00	0.00	0.00	
	2	0				0	0.00				0	0				0	0.00			
	3	0				0	0.00				0	0				0	0.00			
	4	0				0	0.00				0	0				0	0.00			

COPPER SPIKES

H. azteca - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	86.67	86.67	0.00	0.00	0.429	0.41	0.03	8.20
	2	86.67				0.382			
15	1	93.33	93.33	0.00	0.00	0.35	0.35	0.00	0.20
	2	93.33				0.351			
27	1	93.33	96.67	4.72	4.88	0.347	0.34	0.00	1.03
	2	100				0.342			
48	1	66.67	66.67	0.00	0.00	0.317	0.36	0.06	15.85
	2	66.67				0.397			
150	1	20	46.67	37.71	80.81	0.107	0.10	0.01	14.58
	2	73.33				0.087			
268	1	0	0	0	-	-	-	-	-
	2	0				-			

H. azteca - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	86.67	86.67	0.00	0.00	0.466	0.40	0.09	21.50
	2	86.67				0.343			
15	1	86.67	93.34	9.43	10.10	0.413	0.37	0.06	15.38
	2	100				0.332			
27	1	93.33	90.00	4.71	5.23	0.347	0.36	0.02	6.60
	2	86.67				0.381			
48	1	100	93.34	9.43	10.10	0.377	0.33	0.07	21.87
	2	86.67				0.276			
150	1	40	30.00	14.14	47.14	0.115	0.15	0.05	33.72
	2	20				0.187			
268	1	0	0	0	-	-	-	-	-
	2	0				-			

H. azteca - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	86.67	90.00	4.71	5.23	0.372	0.39	0.03	7.39
	2	93.33				0.413			
15	1	93.33	96.67	4.72	4.88	0.373	0.38	0.01	3.69
	2	100				0.393			
27	1	86.67	86.67	0.00	0.00	0.318	0.37	0.07	18.55
	2	86.67				0.414			
48	1	93.33	90.00	4.71	5.23	0.311	0.34	0.05	13.38
	2	86.67				0.376			
150	1	33.33	60.00	37.72	62.86	0.15	0.14	0.01	5.89
	2	86.67				0.138			
268	1	0	0	0	-	-	-	-	-
	2	0				-			

COPPER SPIKES

***C. riparius* - Test 1**

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	86.67	83.34	4.72	5.66	0.332	0.31	0.03	10.53
	2	80				0.286			
15	1	86.67	90.00	4.71	5.23	0.352	0.31	0.06	19.42
	2	93.33				0.267			
27	1	86.67	90.00	4.71	5.23	0.335	0.30	0.05	16.24
	2	93.33				0.266			
48	1	86.67	83.34	4.72	5.66	0.283	0.33	0.07	20.69
	2	80				0.38			
150	1	86.67	86.67	0.00	0.00	0.165	0.15	0.02	10.60
	2	86.67				0.142			
268	1	93.33	86.67	9.43	10.88	0.142	0.12	0.04	33.20
	2	80				0.088			
483	1	26.67	23.34	4.72	20.21	0.035	0.05	0.02	47.14
	2	20				0.07			
1502	1	0	0.00	0.00		-	-	-	-
	2	0				-			

***C. riparius* - Test 2**

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	93.33	80.00	18.85	23.56	0.312	0.40	0.13	31.93
	2	66.67				0.494			
15	1	73.33	76.67	4.72	6.15	0.369	0.33	0.06	18.16
	2	80				0.285			
27	1	93.33	93.33	-	-	0.281	0.28	-	-
	2					-			
48	1	73.33	73.33	-	-	-	0.29	-	-
	2					0.29			
150	1	80	80.00	-	-	-	0.16	-	-
	2					0.155			
268	1		66.67	-	-	-	0.12	-	-
	2	66.67				0.123			
483	1	60	43.34	23.57	54.39	0.052	0.07	0.03	36.37
	2	26.67				0.088			
1502	1	0	0.00	0.00		-	-	-	-
	2	0				-			

***C. riparius* - Test 3**

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	93.33	93.33	0.00	0.00	0.195	0.20	0.00	0.36
	2	93.33				0.196			
15	1	86.67	90.00	4.71	5.23	0.209	0.20	0.02	10.15
	2	93.33				0.181			
27	1	86.67	90.00	4.71	5.23	0.172	0.18	0.02	9.58
	2	93.33				0.197			
48	1	86.67	90.00	4.71	5.23	0.195	0.19	0.01	4.88
	2	93.33				0.182			
150	1	93.33	93.33	0.00	0.00	0.138	0.14	0.00	3.50
	2	93.33				0.145			
268	1	40	43.34	4.72	10.88	0.09	0.10	0.02	18.45
	2	46.67				0.117			
483	1	26.67	26.67	0.00	0.00	0.018	0.03	0.02	57.96
	2	26.67				0.043			
1502	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

COPPER SPIKES

Hexagenia spp. - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	3.925	3.93	0.00	0.13
	2	100				3.932			
13	1	100	100.00	0.00	0.00	3.968	3.97	0.00	0.09
	2	100				3.973			
27	1	100	100.00	0.00	0.00	1.952	1.84	0.16	8.45
	2	100				1.732			
134	1	40	30.00	14.14	47.14	-0.505	-0.42	0.12	-28.82
	2	20				-0.334			
268	1	10	5.00	7.07	141.42	-0.414	-0.41	-	-
	2	0				-			
1342	1	0	0.00	0.00	-	-	-	-	-
	2	0							

Hexagenia spp. - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	3.38	3.47	0.12	3.47
	2	100				3.55			
13	1	100	100.00	0.00	0.00	3.282	3.37	0.13	3.71
	2	100				3.459			
27	1	100	100.00	0.00	0.00	2.521	2.69	0.23	8.71
	2	100				2.852			
134	1	50	30.00	28.28	94.28	-0.272	-0.17	0.15	-88.23
	2	10				-0.063			
268	1	0	5.00	7.07	141.42	-	-0.62	-	-
	2	10				-0.62			
1342	1	0	0.00	0.00	-	-	-	-	-
	2	0							

Hexagenia spp. - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	3.654	3.48	0.24	7.01
	2	100				3.309			
13	1	90	95.00	7.07	7.44	2.97	3.37	0.57	16.80
	2	100				3.771			
27	1	100	100.00	0.00	0.00	2.551	2.72	0.24	8.84
	2	100				2.891			
134	1	30	25.00	7.07	28.28	-0.644	-0.60	0.06	-10.24
	2	20				-0.557			
268	1	0	5.00	7.07	141.42	-0.736	-0.74	-	-
	2	10				-			
1342	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

COPPER SPIKES

T. tubifex - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100.00	0.00	0.00	26	6.50	7.63	1.59	20.87	16	61.54	62.20	0.93	1.50	75	18.75	23.25	6.36	27.37
	2	100				35	8.75				22	62.86				111	27.75			
13	1	100	100.00	0.00	0.00	41	10.25	9.38	1.24	13.20	22	53.66	57.71	5.73	9.93	81	20.25	23.88	5.13	21.47
	2	100				34	8.50				21	61.76				110	27.50			
27	1	100	100.00	0.00	0.00	31	7.75	8.50	1.06	12.48	15	48.39	53.92	7.83	14.52	79	19.75	22.88	4.42	19.32
	2	100				37	9.25				22	59.46				104	26.00			
134	1	100	100.00	0.00	0.00	41	10.25	10.75	0.71	6.58	26	63.41	61.71	2.41	3.91	156	39.00	40.13	1.59	3.97
	2	100				45	11.25				27	60.00				165	41.25			
268	1	100	87.50	17.68	20.20	30	7.50	5.89	2.27	38.57	10	33.33	26.67	9.43	35.36	48	12.00	7.86	5.86	74.57
	2	75				15	4.29				3	20.00				13	3.71			
1342	1	0	0.00	0.00	#DIV/0!	0	0.00	0.25	0.35	141.42	0	-	0.00	-	-	0	0.00	0.00	0.00	-
	2	0				1	0.50				0	0.00				0	0.00			

T. tubifex - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100.00	0.00	0.00	44	11.00	10.00	1.41	14.14	24	54.55	59.22	6.61	11.16	91	22.75	21.25	2.12	9.98
	2	100				36	9.00				23	63.89				79	19.75			
13	1	100	100.00	0.00	0.00	36	9.00	9.38	0.53	5.66	22	61.11	62.61	2.12	3.38			21.25	-	-
	2	100				39	9.75				25	64.10				85	21.25			
27	1	100	100.00	0.00	0.00	38	9.50	9.50	0.00	0.00	24	63.16	60.53	3.72	6.15	80	20.00	21.13	1.59	7.53
	2	100				38	9.50				22	57.89				89	22.25			
134	1	100	100.00	0.00	0.00	41	10.25	10.63	0.53	4.99	25	60.98	60.03	1.33	2.22	124	31.00	31.88	1.24	3.88
	2	100				44	11.00				26	59.09				131	32.75			
268	1	100	100.00	0.00	0.00	17	4.25	4.88	0.88	18.13	1	5.88	9.76	5.48	56.18	6	1.50	1.75	0.35	20.20
	2	100				22	5.50				3	13.64				8	2.00			
1342	1	0	0.00	0.00	-	0	0.00	0.00	0.00	-	0	-	-	-	-	0	0.00	0.00	0.00	-
	2	0				0	0.00				0	-				0	0.00			

T. tubifex - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100.00	0.00	0.00	34	8.50	8.63	0.18	2.05	22	64.71	60.92	5.35	8.78	68	17.00	17.25	0.35	2.05
	2	100				35	8.75				20	57.14				70	17.50			
13	1	100	100.00	0.00	0.00	44	11.00	10.50	0.71	6.73	24	54.55	58.38	5.43	9.30	70	15.56	13.65	2.69	19.71
	2	100				45	10.00				28	62.22				47	11.75			
27	1	100	100.00	0.00	0.00	37	9.25	9.13	0.18	1.94	24	64.86	58.82	8.55	14.53	61	15.25	16.50	1.77	10.71
	2	100				36	9.00				19	52.78				71	17.75			
134	1	100	100.00	0.00	0.00	38	9.50	10.63	1.59	14.97	24	63.16	54.98	11.56	21.03	138	34.50	35.00	0.71	2.02
	2	100				47	11.75				22	46.81				142	35.50			
268	1	100	100.00	0.00	0.00	37	9.25	9.63	0.53	5.51	13	35.14	41.32	8.74	21.16	25	6.25	11.88	7.95	66.99
	2	100				40	10.00				19	47.50				70	17.50			
1342	1	0	0.00	0.00	-	0	0.00	0.00	0.00	-	0	-	-	-	-	0	0.00	0.00	0.00	-
	2	0				0	0.00				0	-				0	0.00			

NICKEL SPIKES

H. azteca -*Test 1*

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	93.33	90.00	4.71	5.23	0.413	0.39	0.04	9.89
	2	86.67				0.359			
23	1	100	93.34	9.43	10.10	0.276	0.29	0.02	8.43
	2	86.67				0.311			
40	1	80	80.00	0.00	0.00	0.293	0.26	0.05	19.19
	2	80				0.223			
73	1	6.67	6.67	0.00	0.00	0.18	0.16	0.04	22.81
	2	6.67				0.13			
227	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
405	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

H. azteca - *Test 2*

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	80	90.00	14.14	15.71	0.359	0.34	0.03	8.34
	2	100				0.319			
23	1	86.67	93.34	9.43	10.10	0.38	0.34	0.05	15.48
	2	100				0.305			
40	1	86.67	86.67	0.00	0.00	0.273	0.31	0.05	15.46
	2	86.67				0.34			
73	1	53.33	66.67	18.86	28.29	0.241	0.21	0.05	22.83
	2	80				0.174			
227	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
405	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

H. azteca - *Test 3*

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	93.34	9.43	10.10	0.343	0.36	0.02	6.87
	2	86.67				0.378			
23	1	93.33	90.00	4.71	5.23	0.289	0.25	0.06	23.71
	2	86.67				0.206			
40	1	93.33	90.00	4.71	5.23	0.321	0.33	0.01	3.65
	2	86.67				0.338			
73	1	80	80.00	0.00	0.00	0.293	0.30	0.01	3.53
	2	80				0.308			
227	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
405	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

NICKEL SPIKES

C. riparius - Test 1

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	93.33	93.33	0.00	0.00	0.329	0.325	0.01	1.96
	2	93.33				0.32			
23	1	100	96.67	4.72	4.88	0.343	0.370	0.04	10.14
	2	93.33				0.396			
40	1	100	100.00	0.00	0.00	0.354	0.354	0.00	0.20
	2	100				0.353			
73	1	93.33	96.67	4.72	4.88	0.36	0.360	0.00	0.20
	2	100				0.359			
227	1	100	96.67	4.72	4.88	0.295	0.295	0.00	0.24
	2	93.33				0.294			
405	1	93.33	93.33	0.00	0.00	0.2	0.228	0.04	17.09
	2	93.33				0.255			
728	1	-	93.33	-	-	-	0.187	-	-
	2	93.33				0.187			
2266	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

C. riparius - Test 2

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	0.393	0.372	0.03	7.98
	2	100				0.351			
23	1	100	93.34	9.43	10.10	0.36	0.387	0.04	9.70
	2	86.67				0.413			
40	1	100	100.00	0.00	0.00	0.332	0.345	0.02	5.33
	2	100				0.358			
73	1	93.33	96.67	4.72	4.88	0.307	0.340	0.05	13.54
	2	100				0.372			
227	1	86.67	86.67	0.00	0.00	0.207	0.232	0.04	15.24
	2	86.67				0.257			
405	1	93.33	96.67	4.72	4.88	0.181	0.170	0.02	9.15
	2	100				0.159			
728	1	93.33	90.00	4.71	5.23	0.149	0.169	0.03	16.74
	2	86.67				0.189			
2266	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

C. riparius - Test 3

Nominal CONC	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	80	90.00	14.14	15.71	0.354	0.338	0.02	6.69
	2	100				0.322			
23	1	100	96.67	4.72	4.88	0.3	0.308	0.01	3.67
	2	93.33				0.316			
40	1	93.33	86.67	9.43	10.88	0.331	0.352	0.03	8.44
	2	80				0.373			
73	1	93.33	86.67	9.43	10.88	0.274	0.311	0.05	16.83
	2	80				0.348			
227	1	86.67	93.34	9.43	10.10	0.286	0.275	0.02	5.92
	2	100				0.263			
405	1	93.33	90.00	4.71	5.23	0.208	0.187	0.03	15.88
	2	86.67				0.166			
728	1	100	83.34	23.57	28.28	0.151	0.107	0.06	58.15
	2	66.67				0.063			
2266	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			

NICKEL SPIKES

Hexagenia spp. - Test 1

Conc	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	5.273	5.18	0.08	1.60
	2	100				5.16			
	3	100				5.112			
40	1	100	80.00	20.00	25.00	5.41	5.59	0.31	5.56
	2	60				5.945			
	3	80				5.404			
130	1	100	100.00	0.00	0.00	3.512	3.57	0.49	13.64
	2	100				3.119			
	3	100				4.088			
405	1	100	86.67	11.55	13.32	0.496	1.24	0.67	53.92
	2	80				1.443			
	3	80				1.792			
1295	1	20	20.00	0.00	0.00	-0.313	-0.40	0.11	-26.32
	2	20				-0.519			
	3	20				-0.374			
4047	1	0	0.00	0.00					
	2	0							
	3	0							

Hexagenia spp. - Test 2

Conc	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	3.927	3.86	0.10	2.49
	2	100				3.791			
40	1	100	100.00	0.00	0.00	3.93	3.85	0.12	3.05
	2	100				3.764			
130	1	100	100.00	0.00	0.00	2.508	2.56	0.07	2.63
	2	100				2.603			
405	1	90	95.00	7.07	7.44	0.609	0.61	0.01	0.94
	2	100				0.601			
1295	1	20	10.00	14.14	141.42	-0.201	-0.20		
	2	0							
4047	1	0	0.00	0.00					
	2	0							

Hexagenia spp. - Test 3

Conc	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
0	1	100	100.00	0.00	0.00	3.492	3.49	0.01	0.28
	2	100				3.478			
40	1	100	100.00	0.00	0.00	3.142	3.14	0.01	0.23
	2	100				3.132			
130	1	90	95.00	7.07	7.44	2.525	2.59	0.10	3.71
	2	100				2.661			
405	1	90	90.00	0.00	0.00	0.214	0.30	0.12	40.20
	2	90				0.384			
1295	1	10	5.00	7.07	141.42	-0.207	-0.21		
	2	0							
4047	1	0	0.00	0.00					
	2	0							

NICKEL SPIKES

***T. tubifex* - Test 1**

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100	0	0	32	8.0	8.20	0.45	5.45	21	65.63	64.03	1.56	2.44	42	10.50	10.70	1.71	15.97
	2	100				32	8.0				20	62.50				35	8.75			
	3	100				32	8.0				20	62.50				38	9.50			
	4	100				32	8.0				21	65.63				47	11.75			
	5	100				36	9.0				23	63.89				52	13.00			
40	1	100	100	0	0	35	8.8	8.60	0.29	3.31	23	65.71	60.55	6.69	11.04	40	10.00	11.38	1.48	13.00
	2	100				36	9.0				20	55.56				41	10.25			
	3	100				34	8.5				19	55.88				52	13.00			
	4	100				33	8.3				23	69.70				49	12.25			
	5	100				34	8.5				19	55.88								
130	1	100	100	0	0	29	7.3	7.80	0.54	6.95	13	44.83	53.76	6.72	12.50	44	11.00	10.10	1.21	11.95
	2	100				30	7.5				17	56.67				35	8.75			
	3	100				34	8.5				20	58.82				46	11.50			
	4	100				33	8.3				16	48.48				36	9.00			
	5	100				30	7.5				18	60.00				41	10.25			
405	1	100	100	0	0	42	10.5	9.50	1.36	14.29	23	54.76	52.96	5.69	10.74	31	7.75	16.00	7.42	46.40
	2	100				34	8.5				19	55.88				59	14.75			
	3	100				32	8.0				17	53.13				41	10.25			
	4	100				37	9.3				16	43.24				91	22.75			
	5	100				45	11.3				26	57.78				98	24.50			
1295	1	100	100	0	0	16	4.0	4.50	0.59	13.03	7	43.75	48.93	18.47	37.75	13	3.25	3.40	1.21	35.49
	2	100				21	5.3				11	52.38				14	3.50			
	3	100				20	5.0				10	50.00				19	4.75			
	4	100				16	4.0				12	75.00				16	4.00			
	5	100				17	4.3				4	23.53				6	1.50			
4050	1	0	0	0		0	0.0	0.00	0.00		0.00	0.00	0.00	0.00		0	0.00	0.00	0.00	
	2	0				0	0.0				0	0.00				0	0.00			
	3	0				0	0.0				0	0.00				0	0.00			
	4	0				0	0.0				0	0.00				0	0.00			
	5	0				0	0.0				0	0.00				0	0.00			

NICKEL SPIKES

***T. tubifex* - Test 2**

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100	0	0	42	10.5	10.50	0.00	0.00	25	59.52	59.52	0.00	0.00	82	20.50	20.50	0.00	0.00
	2	100				42	10.5				25	59.52				82	20.50			
40	1	100	100	0	0	37	9.3	9.75	0.71	7.25	20	54.05	57.51	4.89	8.51	64	16.00	18.75	3.89	20.74
	2	100				41	10.3				25	60.98				86	21.50			
130	1	100	100	0	0	42	10.5	10.00	0.71	7.07	24	57.14	57.52	0.53	0.92	89	22.25	21.75	0.71	3.25
	2	100				38	9.5				22	57.89				85	21.25			
405	1	100	100	0	0	44	11.0	11.50	0.71	6.15	26	59.09	60.80	2.41	3.97	141	35.25	40.38	7.25	17.95
	2	100				48	12.0				30	62.50				182	45.50			
1295	1	100	100	0	0	13	3.3	2.25	1.41	62.85	3	23.08	21.54	2.18	10.10	2	0.50	1.38	1.24	90.00
	2	100				5	1.3				1	20.00				9	2.25			
4050	1	0	0	0		0	0.0	0.00	0.00		0	0.00	0.00	0.00		0	0.00	0.00	0.00	
	2	0				0	0.0				0	0.00				0	0.00			

***T. tubifex* - Test 3**

Nominal CONC	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
0	1	100	100	0	0	34	8.5	8.63	0.18	2.05	19	55.88	59.37	4.93	8.31	49	12.25	13.25	1.41	10.67
	2	100				35	8.8				22	62.86				57	14.25			
40	1	100	100	0	0	37	9.3	9.25	0.00	0.00	23	62.16	58.11	5.73	9.87	68	17.00	16.38	0.88	5.40
	2	100				37	9.3				20	54.05				63	15.75			
130	1	100	100	0	0	37	9.3	8.50	1.06	12.48	24	64.86	61.46	4.81	7.82	84	21.00	17.75	4.60	25.89
	2	100				31	7.8				18	58.06				58	14.50			
405	1	100	100	0	0	37	9.3	9.75	0.71	7.25	18	48.65	51.15	3.54	6.93	104	26.00	30.13	5.83	19.36
	2	100				41	10.3				22	53.66				137	34.25			
1295	1	100	100	0	0	7	1.8	2.25	0.71	31.43	4	57.14	51.30	8.26	16.11	7	1.75	2.25	0.71	31.43
	2	100				11	2.8				5	45.45				11	2.75			
4050	1	0	0	0		0	0.0	0.00	0.00		0	0.00	0.00	0.00		0	0.00	0.00	0.00	
	2	0				0	0.0				0	0.00				0	0.00			

Appendix C

Raw Data for Field-Collected Sediment Tests

Hyalella azteca COLLINGWOOD HARBOUR

Site	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
C6	1	100	80.00	19.44	24.30	0.485	0.332	0.161	48.360
	2	53.33				0.116			
	3	66.67				0.432			
	4	93.33				0.421			
	5	86.67				0.208			
C7	1	53.33	77.33	22.41	28.98	0.18	0.310	0.088	28.482
	2	100				0.339			
	3	93.33				0.27			
	4	53.33				0.351			
	5	86.67				0.411			
C8	1	46.67	66.67	27.22	40.82	0.317	0.232	0.213	91.750
	2								
	3	93.33				0.493			
	4	40				0.052			
	5	86.67				0.065			
C9	1	53.33	78.34	16.67	21.28	0.25	0.244	0.068	28.045
	2	86.67				0.283			
	3								
	4	86.67				0.298			
	5	86.67				0.146			
C10	1	86.67	95.00	6.38	6.72	0.115	0.206	0.094	45.635
	2								
	3	100				0.312			
	4	93.33				0.14			
	5	100				0.258			
C11	1	86.67	92.00	8.69	9.45	0.215	0.301	0.149	49.580
	2	80				0.15			
	3	100				0.218			
	4	100				0.441			
	5	93.33				0.481			
Lab Control	1	93.33	93.33	5.44	5.83	0.22	0.331	0.100	30.219
	2	100				0.36			
	3	86.67				0.414			
	4	93.33							

Chironomus riparius COLLINGWOOD HARBOUR

Site	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
C6	1	93.33	95.00	3.34	3.51	0.201	0.188	0.037	19.897
	2								
	3	100				0.198			
	4	93.33				0.22			
	5	93.33				0.134			
C7	1	80	85.33	8.69	10.19	0.167	0.209	0.033	15.927
	2	80				0.254			
	3	80				0.229			
	4	86.67				0.194			
	5	100				0.203			
C8	1	86.67	61.67	33.28	53.97	0.27	0.185	0.089	47.996
	2	13.33				0.06			
	3	80				0.204			
	4								
	5	66.67				0.206			
C9	1		70.00	12.77	18.24		0.278	0.091	32.824
	2	66.67				0.363			
	3	53.33				0.156			
	4	80				0.329			
	5	80				0.263			
C10	1	80	85.33	7.30	8.56	0.293	0.316	0.019	5.886
	2	80				0.319			
	3	93.33				0.304			
	4	93.33				0.325			
	5	80				0.341			
C11	1	93.33	90.67	11.16	12.30	0.224	0.282	0.037	12.981
	2	86.67				0.288			
	3	100				0.28			
	4	100				0.292			
	5	73.33				0.325			
Lab Control	1	66.67	86.67	12.47	14.39	0.313	0.307	0.028	9.268
	2	86.67				0.353			
	3	93.33				0.281			
	4	86.67				0.288			
	5	100				0.3			

Hexagenia spp. COLLINGWOOD HARBOUR

Site	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
C6	1	100	100.00	0.00	0.00	6.49	6.281	0.788	12.543
	2	100				5.779			
	3	100				7.222			
	4	100				6.695			
	5	100				5.218			
C7	1	90	98.00	4.47	4.56	4.718	4.767	1.551	32.532
	2	100				5.048			
	3	100				2.222			
	4	100				6.363			
	5	100				5.484			
C8	1	100	100.00	0.00	0.00	2.798	5.803	2.266	39.051
	2	100				5.766			
	3	100				5.638			
	4	100				5.626			
	5	100				9.187			
C9	1	80	96.00	8.94	9.32	0.633	2.761	1.796	65.066
	2	100				1.322			
	3	100				5.048			
	4	100				3.04			
	5	100				3.761			
C10	1	100	98.00	4.47	4.56	1.81	4.368	1.557	35.649
	2	100				4.644			
	3	90				5.982			
	4	100				4.348			
	5	100				5.057			
C11	1	100	100.00	0.00	0.00	0.367	3.384	1.747	51.636
	2	100				3.404			
	3	100				4.112			
	4	100				4.57			
	5	100				4.468			
Lab Control	1	100	100.00	0.00	0.00	7.203	6.651	0.384	5.771
	2	100				6.618			
	3	100				6.448			
	4	100				6.183			
	5	100				6.804			

Tubifex tubifex COLLINGWOOD HARBOUR

Site	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
C6	1	100	95.00	11.18	11.77	41	10.25	10.24	0.66	6.44	3	7.32	14.91	7.80	52.33	0	0.00	2.21	2.22	100.37
	2	100				39	9.75				5	12.82				18	4.50			
	3	100				43	10.75				12	27.91				1	0.25			
	4	75				33	9.43				5	15.15				16	4.57			
	5	100				44	11.00				5	11.36				7	1.75			
C7	1	100	100.00	0.00	0.00	31	7.75	9.75	1.61	16.52	4	12.90	16.01	5.38	33.59	0	0.00	0.10	0.14	136.93
	2	100				43	10.75				9	20.93				1	0.25			
	3	100				33	8.25				4	12.12				0	0.00			
	4	100				44	11.00				5	11.36				0	0.00			
	5	100				44	11.00				10	22.73				1	0.25			
C8	1	100	100.00	0.00	0.00	39	9.75	10.75	0.59	5.45	9	23.08	24.62	9.69	39.35	38	9.50	11.20	4.90	43.78
	2	100				45	11.25				11	24.44				73	18.25			
	3	100				43	10.75				11	25.58				21	5.25			
	4	100				44	11.00				17	38.64				54	13.50			
	5	100				44	11.00				5	11.36				38	9.50			
C9	1	100	100.00	0.00	0.00	37	9.25	9.15	0.63	6.86	8	21.62	21.96	9.40	42.80	1	0.25	4.30	5.59	129.90
	2	100				40	10.00				15	37.50				55	13.75			
	3	100				33	8.25				5	15.15				0	0.00			
	4	100				37	9.25				8	21.62				15	3.75			
	5	100				36	9.00				5	13.89				15	3.75			
C10	1	100	100.00	0.00	0.00	38	9.50	7.80	4.42	56.72	3	7.89	16.03	12.95	80.76	4	1.00	2.70	2.92	108.26
	2	100				36	9.00				9	25.00				24	6.00			
	3	100					0.00										0.00			
	4	100				44	11.00				1	2.27				3	0.75			
	5	100				38	9.50				11	28.95				23	5.75			
C11	1	100	95.00	11.18	11.77	36	9.00	8.81	1.38	15.71	11	30.56	38.48	10.51	27.30	47	11.75	10.31	3.49	33.83
	2	100				27	6.75				12	44.44				44	11.00			
	3	100				33	8.25				8	24.24				30	7.50			
	4	100				39	9.75				19	48.72				60	15.00			
	5	75				36	10.29				16	44.44				22	6.29			
Lab Control	1	100	100.00	0.00	0.00	40	10.00	10.63	0.92	8.70	22	55.00	52.04	7.39	14.19	137	34.25	33.31	2.22	6.67
	2	100				39	9.75				16	41.03				127	31.75			
	3	100				47	11.75				26	55.32				144	36.00			
	4	100				44	11.00				25	56.82				125	31.25			

Hyalella azteca ROUYN-NORANDA

Lake	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
Opasatica	1	60	25.33	21.81	86.08	0.2	0.323	0.156	48.294
	2	26.67				0.525			
	3	0							
	4	20				0.367			
	5	20				0.2			
Vaudray	1	13.33	40.00	25.39	63.47	0.15	0.139	0.013	9.121
	2	66.67				0.12			
	3	66.67				0.15			
	4	33.33				0.14			
	5	20				0.133			
Joannes	1	0	32.00	32.46	101.42		0.235	0.109	46.398
	2	6.67				0.1			
	3	66.67				0.35			
	4	20				0.2			
	5	66.67				0.289			
D'Alembert	1	93.33	86.67	7.70	8.88	0.179	0.193	0.042	21.796
	2	93.33				0.15			
	3	80				0.192			
	4	80				0.25			
	5								
Default	1	0	0.00	0.00	-	-	-	-	-
	2	0				-			
	3	0				-			
	4	0				-			
	5	0				-			
Lab Control	1	100	98.67	2.98	3.02	0.313	0.335	0.047	13.913
	2	100				0.28			
	3	100				0.38			
	4	100				0.387			
	5	93.33				0.314			

Chironomus riparius ROUYN-NORANDA

Lake	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
Opasatica	1	46.67	74.67	15.92	21.32	0.571	0.402	0.098	24.318
	2	80				0.383			
	3	80				0.383			
	4	80				0.35			
	5	86.67				0.323			
Vaudray	1	46.67	54.67	7.30	13.36	0.514	0.388	0.169	43.440
	2	53.33				0.1			
	3	53.33				0.438			
	4	53.33				0.5			
	5	66.67				0.39			
Joannes	1	100	58.67	33.47	57.04	0.353	0.414	0.119	28.772
	2	20				0.6			
	3	86.67				0.462			
	4	46.67				0.357			
	5	40				0.3			
D'Alembert	1	86.67	85.34	2.98	3.50	0.4	0.309	0.131	42.277
	2	80				0.492			
	3	86.67				0.215			
	4	86.67				0.192			
	5	86.67				0.246			
Default	1	40	76.00	21.91	28.83	0.133	0.108	0.032	29.140
	2	93.33				0.093			
	3	80				0.075			
	4	93.33				0.15			
	5	73.33				0.091			
Lab Control	1	86.67	85.33	8.69	10.19	0.369	0.325	0.050	15.475
	2	73.33				0.372			
	3	93.33				0.25			
	4	93.33				0.307			
	5	80				0.325			

Hexagenia spp. ROUYN-NORANDA

Lake	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
Opasatica	1	100	94.00	13.42	14.27	2.119	2.125	0.121	5.696
	2	100				2.215			
	3	70				2.221			
	4	100				1.923			
	5	100				2.146			
Vaudray	1	100	100.00	0.00	0.00	3.812	3.881	0.330	8.493
	2	100				3.493			
	3	100				3.73			
	4	100				3.997			
	5	100				4.374			
Joannes	1	100	100.00	0.00	0.00	2.966	3.080	0.306	9.942
	2	100				3.464			
	3	100				3.23			
	4	100				3.1			
	5	100				2.642			
D'Alembert	1	100	96.00	8.94	9.32	3.931	3.351	0.456	13.606
	2	100				3.734			
	3	80				3.178			
	4	100				2.896			
	5	100				3.017			
Default	1	100	78.00	16.43	21.07	-0.208	-0.346	0.195	-56.294
	2	60				-0.602			
	3	70				-0.114			
	4	90				-0.344			
	5	70				-0.46			
Lab Control	1	100	97.50	5.00	5.13	5.491	5.308	0.458	8.630
	2	100				5.322			
	3								
	4	90				4.673			
	5	100				5.747			

Tubifex tubifex **ROUYN-NORANDA**

Lake	Rep	Survival	Mean	SD	CV	Total Cocoons	Cocoon Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
Opasatica	1	75	95.00	11.18	11.77	32	9.14	8.08	1.44	17.87	20	62.50	69.59	10.23	14.70	56	16.00	12.95	5.89	45.51
	2	100				30	7.50				21	70.00				18	4.50			
	3	100				26	6.50				18	69.23				43	10.75			
	4	100				29	7.25				25	86.21				53	13.25			
	5	100				40	10.00				24	60.00				81	20.25			
Vaudray	1	100	100.00	0.00	0.00	39	9.75	9.80	0.51	5.23	23	58.97	54.03	3.20	5.93	62	15.50	15.80	4.68	29.63
	2	100				39	9.75				21	53.85				91	22.75			
	3	100				41	10.25				22	53.66				70	17.50			
	4	100				36	9.00				18	50.00				43	10.75			
	5	100				41	10.25				22	53.66				50	12.50			
Joannes	1	100	100.00	0.00	0.00	33	8.25	9.10	0.96	10.57	22	66.67	54.77	9.47	17.29	81	20.25	20.35	5.94	29.21
	2	100				32	8.00				18	56.25				85	21.25			
	3	100				39	9.75				16	41.03				47	11.75			
	4	100				41	10.25				24	58.54				114	28.50			
	5	100				37	9.25				19	51.35				80	20.00			
D'Alembert	1	100	100.00	0.00	0.00	46	11.50	10.00	1.22	12.25	28	60.87	58.14	4.62	7.95	77	19.25	20.70	4.38	21.15
	2	100				42	10.50				23	54.76				112	28.00			
	3	100				36	9.00				23	63.89				68	17.00			
	4	100				34	8.50				20	58.82				72	18.00			
	5	100				42	10.50				22	52.38				85	21.25			
Default	1	75	80.00	20.92	26.15	27	7.71	6.67	1.31	19.65	27	100.00	100.00	0.00	0.00	7	2.00	5.86	4.47	76.21
	2	75				22	6.29				22	100.00				46	13.14			
	3	100				23	5.75				23	100.00				27	6.75			
	4	100				21	5.25				21	100.00				11	2.75			
	5	50				25	8.33				25	100.00				14	4.67			
Lab Control	1	100	100.00	0.00	0.00	45	9.00	9.90	1.24	12.58	25	55.56	55.75	4.06	7.28	114	22.80	27.71	3.82	13.79
	2	100				36	9.00				20	55.56				100	25.00			
	3	100				40	10.00				21	52.50				117	29.25			
	4	100				48	12.00				30	62.50				130	32.50			
	5	100				38	9.50				20	52.63				116	29.00			

Hyalella azteca SUDBURY

Site	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
S1	1	66.67	22.67	31.83	140.44	0.04	0.035	0.008	22.545
	2	0							
	3	0							
	4	0							
	5	46.67				0.029			
S3	1		51.67	23.96	46.38		0.116	0.107	92.612
	2	86.67				0.123			
	3	40				0.083			
	4	46.67				0.257			
	5	33.33				0			
S2	1	40	8.00	17.89	223.61	0.017	0.017		
	2	0							
	3	0							
	4	0							
	5	0							
S4	1	0	0.00	0.00					
	2	0							
	3	0							
	4	0							
	5	0							
Lab Control	1	100	98.67	2.98	3.02	0.247	0.236	0.0856	36.264
	2	100				0.28			
	3	93.33				0.2			
	4	100				0.34			
	5	100				0.113			

Chironomus riparius SUDBURY

Site	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
S1	1	93.33	88.33	11.39	12.89	0.4	0.412	0.083	20.204
	2								
	3	73.33				0.3			
	4	100				0.487			
	5	86.67				0.462			
S3	1	86.67	89.33	7.60	8.51	0.485	0.438	0.056	12.721
	2	93.33				0.436			
	3	80				0.35			
	4	100				0.487			
	5	86.67				0.431			
S2	1	93.33	80.00	17.00	21.24	0.414	0.356	0.128	35.993
	2	60				0.478			
	3	100				0.38			
	4	80				0.367			
	5	66.67				0.14			
S4	1	66.67	48.00	12.83	26.72	0.09	0.126	0.045	36.196
	2	33.33				0.2			
	3	53.33				0.138			
	4	40				0.1			
	5	46.67				0.1			
Lab Control	1	93.33	90.66	5.96	6.58	0.329	0.247	0.053	21.627
	2	93.33				0.271			
	3	93.33				0.229			
	4	93.33				0.207			
	5	80				0.2			

Hexagenia spp. SUDBURY

Site	Rep	Survival	Mean	SD	CV	Growth	Mean	SD	CV
S1	1	90	96.00	5.48	5.71	4.552	3.463	0.892	25.757
	2	100				3.543			
	3	90				2.062			
	4	100				3.574			
	5	100				3.582			
S3	1	90	96.00	5.48	5.71	1.545	1.974	0.578	29.292
	2	100				2.566			
	3	90				2.642			
	4	100				1.638			
	5	100				1.48			
S2	1	90	84.00	18.17	21.63	0.646	0.289	0.471	162.910
	2	100				0.06			
	3	100				0.918			
	4	60				-0.208			
	5	70				0.031			
S4	1	10	8.00	8.37	104.58	-0.507	-0.669	0.263	-39.381
	2	0							
	3	10				-0.973			
	4	20				-0.527			
	5	0							
Lab Control	1	100	100.00	0.00	0.00	5.001	4.882	0.442	9.044
	2	100				4.957			
	3	100				4.118			
	4	100				5.086			
	5	100				5.249			

Tubifex tubifex **SUDBURY**

Site	Rep	Survival	Mean	SD	CV	Total Cocoons	# Cocoons/ Adult	Mean	SD	CV	Empty Cocoons	% Hatched	Mean	SD	CV	Total Young	# Young/ Adult	Mean	SD	CV
S1	1	100	100.00	0.00	0.00	44	12.57	10.81	1.01	9.30	23	52.27	57.69	3.23	5.60	121	34.57	38.06	4.58	12.03
	2	100				42	10.50				25	59.52				181	45.25			
	3	100				42	10.50				25	59.52				143	35.75			
	4	100				40	10.00				24	60.00				139	34.75			
	5	100				42	10.50				24	57.14				160	40.00			
S3	1	100	100.00	0.00	0.00	45	11.25	11.25	-	-	24	53.33	53.33	-	-	173	43.25	43.25		
	2	100																		
	3	100																		
	4	100																		
	5	100																		
S2	1	100	100.00	0.00	0.00	43	10.75	10.75	0.00	0.00	23	53.49	52.33	1.64	3.14	138	34.50	35.75	1.77	4.94
	2	100																		
	3	100				43	10.75				22	51.16				148	37.00			
	4	100																		
	5	100																		
S4	1	0	15.00	33.54	223.61	0	0.00	0.00	0.00		0					0	0.00	0.57	1.28	223.61
	2	0				0	0.00				0					0	0.00			
	3	75				0	0.00				0					10	2.86			
	4	0				0	0.00				0					0	0.00			
	5	0				0	0.00				0					0	0.00			
Lab Control	1	100	100.00	0.00	0.00	45	11.25	11.80	1.12	9.52	23	51.11	50.07	1.05	2.10	108	27.00	27.90	4.09	14.68
	2	100				44	11.00				22	50.00				95	23.75			
	3	100				51	12.75				25	49.02				139	34.75			
	4	100				43	10.75				22	51.16				110	27.50			
	5	100				53	13.25				26	49.06				106	26.50			