

# **STRUCTURAL AND FUNCTIONAL RESPONSES TO METAL-ENRICHED LEAF MATERIAL IN SMALL STREAMS**

by

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Master of Science

in the

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## Abstract

Depositions of metals from vehicle exhaust and industrial activities impact urban watersheds worldwide. The effects on stream ecosystem function are not fully understood. I addressed this question by considering how the structure and function of stream invertebrate assemblages respond to metal-enriched basal resources. We grew alder (*Alnus rubra*) saplings under three mixed metal treatments corresponding to British Columbia's interim sediment quality guidelines (ISQG) and probable effect levels (PEL) for copper, cadmium and zinc. Triplicate 5g packs of control (no metals), ISQG, and PEL leaves were deployed in six Vancouver area streams. After four weeks, we sampled leaf mass remaining and invertebrate families. Family richness and density were significantly higher in PEL leaf packs. PEL leaf breakdown was significantly faster, potentially a result of lower percent carbon and, therefore, reduced development of structural compounds. These results could have implications to carbon availability and cycling in metal-polluted watersheds.

**Keywords:** organic matter cycling; metal pollution; urban ecology; ecosystem function; stream ecology; British Columbia

## **Dedication**

To my grandmothers— Florence and Jocelyn in gratitude for inspiring and educating me.

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## **Preface**

Metal pollution is a globally important environmental concern, the impacts of which on freshwater ecosystems are not fully understood. Freshwater ecosystems are unique in that they accumulate run-off deposited throughout the watershed, and thus, may be at increased risk of metal exposure. Freshwater ecosystems provide numerous ecological services, such as nutrient cycling, waste decomposition, carbon sequestration, and species habitat. Numerous services, not least of which include human drinking water, can be threatened by decreased ecosystem function. Given a growing interest in ecosystem restoration, and the need to preserve the integrity of freshwater ecosystems, this thesis will review the current usage of plants in phytoremediation of metal-contaminated sites as well as toxicity (section 1). This review then serves to provide the rationale for the current study; the uptake and accumulation of metals by riparian vegetation and the implications of such on the cycling of this organic material in streams (section 2-6).

# 1. Literature Review

The conservation and remediation of freshwater ecosystems are globally important environmental issues. Freshwater ecosystems are particularly susceptible to pollutant deposition and are threatened by growing industrialization and urbanization (Dudgeon et al., 2006). Freshwater is additionally the ultimate receptor of pollution deposited in the watershed, “the gutters down which flow the ruins of continents” (Leopold, 1964), so it is imperative that we understand how pollutants, especially those of global significance, impact aquatic communities and ecosystem function. Given the importance of protecting ecosystems from toxic metal levels, this review will explore sources of metal pollution, metal stress in plants, the use of plants in phytoremediation and the need to better address how metal-contaminated plant material behaves in ecosystems.

## 1.1. Metals in the Environment

Metals are a naturally occurring part of the environment, though their release in biologically available forms has accelerated greatly since the industrial revolution with increased mining, smelting and the by-products of these activities (Marques et al., 2011). While some metals are micronutrients required to support plant and animal life, elevated levels of these metals can be toxic. In contrast, other metals are not required for life and are toxic even at low concentrations (Salt et al., 1995). Urban environments particularly receive contaminants from a variety of sources due to their proximities to different land usages. Vehicle exhaust contains trace metals such as Pb, Zn, Mn, Cu and Cd that can be transported up to several kilometres away from the road (Virard et al. 2004). Traffic density and proximity to roads both correlate positively with soil metal concentration (Virard et al., 2004). Rain events further mobilize metals in urban environments flushing metals that have been deposited on roads and other impervious surfaces into waterways and storm drains (Li et al., 2009). Mountainous regions receive airborne pollutants as

cool air condenses water increasing precipitation at higher altitudes. Contaminants deposited in snow packs may then flush into lower altitude streams with the spring freshet (Daly and Wania 2005). In municipal parks and gardens, pesticides and acidifying fertilizers such as ammonium salts may exacerbate metal leaching from more stable complexes, while phosphate fertilizers may even contain metals such as Cd (Cheraghi et al., 2012). Municipal landfills can leach metals and organic contaminants into the soil and ultimately groundwater (Zhang et al., 2010). The anthropogenically accelerated release of metals can also impact plants and animals in these ecosystems.

## **1.2. Impact of Common Metal Pollutants on Plants**

While some metals (such as Zn, Cu, Mn, Mg, Fe) are essential micronutrients, required for plant growth and metabolism, they can be toxic in excess quantities (Salt et al., 1995). Other metals, such as Cd, Hg and Pb can be toxic even at low levels, have no biological function and can even create deficiencies in essential minerals such as Zn and Cu (Blake and Goulding, 2002). Additionally, combinations of metals can produce additive and synergistic toxicological effects, and as such many factors must be considered in evaluating the toxicity of metals (Hagemeyer, 2004).

There is tremendous variation in uptake capacity and sensitivity to metals between plant species. Plants can be categorized into three major uptake strategies based on the amount of metal taken into above-ground tissues, relative to the soil concentration. Metal excluders transport very little metals to aerial parts, but can accumulate much in their roots. Metal indicators generally reflect soil metal concentration in their tissues, while hyperaccumulators concentrate metals beyond that of the soil (Memon and Schroder, 2009). In geographical locations that are naturally rich in metals, plants may be better adapted to tolerate high metal levels, strategies that will also be mediated by soil pH (Memon and Schroder, 2009). Further understanding of these uptake strategies is valuable information in developing phytoremediation technology and understanding how metal-enriched organic material will behave in ecosystems.

## **1.3. Impact of Metal Pollution on Ecosystem Structure and Function**

Metal pollution can limit the productivity of sensitive species and ecosystem function. The linkage between biodiversity and ecosystem function is the focus of much research (Duarte et al., 2008). Organic matter breakdown is a key functional process and measure of ecosystem integrity in freshwater ecosystems (Gessner and Chauvet, 2002). Urban environments and mining sites are typically exposed to elevated metal concentrations and have been the sites of considerable research on ecosystem function.

### **1.3.1. Mining Studies**

There are numerous documented adverse effects of acid mine drainage on fish, invertebrate, plant and microbial community structure and function (Clements et al., 2010). Within communities, functional shifts depend on the sensitivity of species to the pollution and the redundancy of the network. In Colorado, USA, Clements (1999) found that certain mayflies were more susceptible to predation in metal-contaminated streams. In British Columbia, Marsdesn and DeWreedee (2000) report changes in a macroalgae community, an important measure of productivity in aquatic ecosystems, surrounding the acid mine drainage output in Britannia Beach.

### **1.3.2. Urban Studies**

Urban environments concentrate metals from a variety of sources, and the rate of delivery to waterways is accelerated by increased impervious surface area such as roads, compacted soils and buildings. Impervious surfaces can lead to higher storm flashes, stream bank erosion, higher turbidity and habitat loss (Walsh et al., 2005). Sedimentation can also increase bioavailability of certain metal species (Perin et al., 1997). During storm events, which scour deposits, which may include metals off of road surfaces and mobilize sediments within streams, total dissolved copper and zinc exceeded chronic levels for the protection of aquatic life in urbanized watersheds (Li et al., 2009). This points to the need to examine impacts of intermittent metal exposure on stream communities as well as overall trends in metal deposition. While organic matter decomposition is an important functional indicator of ecosystem health, there are many

intercorrelated factors that influence its rate in urban streams. Chadwick et al. (2007) found a negative relationship between metal level and microbial communities and associated leaf litter breakdown, however, the positive effects of nutrient addition also accelerated breakdown rate. Deforestation in the watershed, even where a buffer is left can also accelerate leaf breakdown (LeCerf and Richardson, 2010). In contrast, Woodcock and Huryn (2004) observed no impact on leaf decomposition along a pollution gradient in Maine due to serial replacement of the dominant shredder species. Similarly, in a transplant experiment of leaves colonized upstream and downstream of an industrial park, Duarte et al. (2008) found reduced fungal diversity but not biomass and consequently no reduction in leaf breakdown rate at the metal-polluted site.

## **1.4. In-situ Remediation Strategies**

Remediation of metal-contaminated sites can be particularly challenging because metal ions cannot be biodegraded or incinerated. Remediation strategies must use the fact that metals can be transformed from one redox state to another and complexed with organic compounds, thus making them less bioavailable in the environment (Marques et al., 2011). In-situ remediation strategies, such as the use of hyperaccumulator plants, are desirable because they avoid large-scale excavation of soil for chemical washing, and attempt to retain some persistent ecosystem features.

### **1.4.1. Chemical Buffering**

Chemical buffering to prevent soil acidification is a precautionary approach to prevent metals from leaching outside of the contaminated area and becoming more bioavailable (Blake and Goulding, 2002). In acidic environments, such as agricultural sites and decommissioned mines, applying lime or alkali salts can reduce metal leaching. However, this may not be practical on larger sites, may require costly reapplications. Additionally, artificial manipulations of soil pH can disrupt the establishment of microbial and plant communities that could aid in the site's restoration (Blake and Goulding, 2002). Therefore, there is great interest in using less destructive techniques in site remediation.

### **1.4.2. Microbial Fixation**

Microorganisms can reduce metal mobility through direct sequestration or by changing the redox state of the metal, allowing it to be taken up by plant roots (Marques et al., 2011). Metals can also be complexed to cellular biomass, or concentrated as exudates within the organism, thus stabilizing them, and reducing potential leaching outside of the contaminated environment (Marques et al., 2011). Chemolithic bacteria, which can increase metal mobility and acidify their environments may be used in conjunction with phytoremediation techniques to enhance plant uptake of toxic metals (Lasat, 2002).

### **1.4.3. Phytoremediation**

Phytoremediation uses plants to sequester, concentrate or immobilize metal contaminants and can be divided into a few major techniques. Phytoextraction uses plants to concentrate metals in harvestable tissues, so that they can later be physically removed from the area. Phytostabilization and rhizofiltration use plants (and their roots) to stabilize and concentrate metals so to reduce their bioavailability within the ecosystem (Salt et al., 1995). Phytoremediation is desirable because it is less invasive than removing sediments for external treatment, does not require the reapplication of buffering agents and other chemicals, and helps restore stability and ecological integrity to the environment. Additionally, it can be up to 1000x cheaper than methods that involve excavation (Memon and Schroder, 2009). Drawbacks include the longer time-scale and completeness of metal removal required, and the potential for metal migration from the immediate environment during that time (Memon and Schroder, 2009).

#### **1.4.3.1. Phytoextraction**

Phytoextraction uses plants to accumulate metals in harvestable tissues, such as leaves, and above-ground roots and shoots, so that the metal-containing tissue can be physically removed from the site. While phytoextraction is less destructive than excavating soil for washing, metals can re-enter the ecosystem through leaf senescence and animal grazing if biomass is not removed. Additionally, the end fate of this biomass must also be considered (Khan et al., 2004). Lettens et al. (2011) monitored metal content of poplar leaves grown on dredged polluted and unpolluted soils over a six-year

period. They found that foliar metal content declined over time in younger stands. Cadmium, zinc and manganese leaf content was stable in older stands grown on contaminated soils. Declining metal content in younger stands could have reflected a decline in growth rate or a decline in soil bioavailable metals, both of which are important mechanisms to phytoremediation (Lettens et al., 2011).

#### **1.4.3.2. Phytostabilization**

Phytostabilization uses plants to reduce the availability of metals in the ecosystem and to reduce the spread of metals outside of the contaminated area. The aim is not to remove biomass, but create a sustaining community of metal-tolerant plants. This technique reduces vertical leaching into groundwater, airborne spread, and availability to soil-dwelling organisms (Salt et al., 1995). Phytostabilization not only stabilizes metals in the ecosystem, but reduces erosion and weathering by providing vegetative cover and root structure to loose, disturbed soils by providing physical structure (Salt et al., 1995). Ideal plant species should be metal-tolerant and fast-growing, with dense roots systems (Prasad et al., 2003). Abandoned mine tailings ponds are a global issue, but much research has been done to identify plant species that can be used in stabilizing metals, and promoting more neutral acidity. Additionally, there is a close relationship between microbial community composition and plant succession (Mendez et al., 2007).

#### **1.4.3.3. Rhizofiltration**

Rhizofiltration uses plant roots to absorb and concentrate metals, typically from aquatic environments and wastewater removed from the site (Prasad et al., 2003). Plants may be grown hydroponically and transported to contaminated water to concentrate metals in the roots, usually over a defined period of optimal growth before being removed (Prasad et al., 2003). Plants with long, rapidly-growing roots and the ability to absorb metals over a long period of time are desirable (Salt et al., 1995). Mishra et al. (2009) found a high removal efficiency of Hg by two aquatic macrophytes *Pistia stratiotes* and *Azolla pinnata*, which effectively reduced the concentration of Hg in mining effluent by 80% once macrophytes were removed. Like phytoextraction, this creates the secondary waste product of metal-enriched biomass, the behaviour of which if left to decompose in the wetland is less studied.

## **1.5. Integrated Implications of Metal Enrichment on Ecosystems**

Research has addressed changes in metal concentration of terrestrial detritus, and lab studies attempt to understand how single species respond to metal-enriched food material, but there is little literature that actually explores the decomposition of metal-contaminated organic material with respect to ecosystem health assessment. In the lab, Godet et al. (2011) highlight the important contribution of metal-contaminated tree litter as a basal food source in forest ecosystems. Godet et al. (2011) performed a feeding choice experiment, providing isopods with leaves containing a gradient of heavy metals, finding a positive correlation between body burden and leaf metal concentration and a negative correlation with feeding and growth rate. Schaller et al. (2011) also found that invertebrate species may actually promote the concentration of metals in detritus, a phenomenon that has been better studied in terrestrial systems. Sundberg et al. (2006) enriched detritus in constructed wetlands to assess toxicity to an amphipod, addressing what happens to metal-enriched leaf material when it is allowed to decompose in the ecosystem. They found that detritus tended to concentrate metals with decomposition, increasing the toxicity of the ecosystem for the decomposer (Sundberg et al., 2006). Likewise, Nelson (2011) measured trace elements Hg, As and Se in aquatic macrophytes before and after decomposition. This study found that trace metals increased slightly with decomposition and that metals from sediment on leaf materials can affect breakdown rate (Nelson, 2011). Weis and Weis (2004) studied the metal content of decomposing plants and the potential deposition of this material into marshes, highlighting the importance of understanding final fate of metals and their potential toxicity in environments, which are largely not understood.

## **1.6. Conclusion**

While phytoremediation can offer cost effective, less environmentally-destructive solutions in the remediation of metal-contaminated sites, there are still gaps in our understanding of the behaviour of contaminated ecosystems particularly with respect to carbon cycling. While delivery of metals to aquatic ecosystems through runoff and direct deposition are better studied, less is known about how metal-enriched organic material

decomposes in aquatic systems. Given the impacts of metal contamination on plant growth and development of structural compounds (Elobeid et al., 2012), decomposition may be accelerated due to physiological changes in the plant structure (Nikula et al., 2010), while high metal concentrations could inhibit the establishment of decomposer communities (Clements et al., 2010). This information could be valuable in managing community establishment, contaminant transport and downstream carbon availability in remediation of contaminated sites.

## 2. Introduction

Freshwater ecosystems provide a suite of invaluable services to many species, including our own. These include nutrient and carbon cycling, habitat, drinking water, food, and industrial and recreational resources (Dudgeon et al., 2006). Urbanization, however, presents a multitude of stresses on freshwater systems, such as reduced riparian areas, increased sedimentation, altered thermal regimes, flashier hydrographs, morphological changes, eutrophication, pollutant exposure, habitat loss and species loss (Chadwick et al., 2007; Walsh et al., 2005). Given our dependence on freshwater resources, understanding how globally important pollutants, such as metals, impact the structure and function of freshwater ecosystems.

Since the industrial revolution, human activities have accelerated the release of biologically-available metals into the environment (Marques et al., 2011). Transportation infrastructure and vehicles are major contributors of metals to urban environments (Davis et al., 2001). Atmospheric deposition of metals shows a predictable declining pattern away from roads (Peachey et al., 2009; Viard et al., 2004). Davis et al. estimate that brake pads shed up to 75  $\mu\text{g}$ / vehicle-km of copper, 89  $\mu\text{g}$ / vehicle-km zinc and 0.05  $\mu\text{g}$ /km-vehicle cadmium, whereas tires can contain 1 g/ kg zinc (2001). The construction of impervious surfaces such as roads, buildings and compacted soils expedite the transport of these materials to urban streams and riparian areas during rain events (Li et al., 2009). Urban stream studies frequently find trace metals exceed public health and water quality standards (Peters, 2009; Brydon et al., 2009). In the greater Vancouver area (British Columbia, Canada), stream sediment metal levels have also been found to exceed the provincial sediment quality guidelines for health and functioning of aquatic ecosystems (Brydon et al., 2009). These factors alone pose a risk to stream community structure and functioning, which may additionally be exacerbated by increased turbidity, reduced dissolved oxygen, and altered hydrology documented in Vancouver-area streams (Finkenbine, 2000).

Metal pollution can additionally have adverse effects on ecosystem function, such as primary productivity and carbon cycling. Leaf litter decomposition is an important measure of ecosystem integrity as allochthonous leaf material frequently forms the base of foodwebs in small streams (Richardson et al., 2005). The effects of urbanization on leaf breakdown are often confounding. While increased water flow, turbidity, and nutrient enrichment may accelerate this process, other factors such as decreased pH and robustness of decomposer communities may slow carbon processing (Chadwick et al., 2007). Since increased metal loads in riparian zones are expected to be reflected in organic matter, metal enrichment is predicted to have two major impacts on organic matter decomposition: 1. reduced development of structural compounds, reduced percent carbon resulting in faster decomposition in stream (Nikula et al., 2010); 2. inhibitory effects on aquatic communities directly associated with the leaves resulting in slower decomposition (Carlisle and Clements, 2005; Godet et al., 2011).

Given the importance of leaf litter as a basal food resource in small streams, we investigated the impact of metal-enrichment on leaf chemistry in red alder (*Alnus rubra*) and the subsequent decomposition of those leaves in streams. We selected red alder for its fast growth and role as an important contributor of allochthonous organic material to many streams in the region. Alder is found in many disturbed riparian habitats in urban British Columbia, so it may be an important plant in local remediation efforts (Villarin et al., 2009; Roy et al., 2007). Since provincial guidelines have an important role in informing development of urban, industrial and mining activities, we will test the effects of these guidelines for safe levels of copper, cadmium and zinc in aquatic sediments on alder leaf growth and then decomposition in urban streams.

In trees treated with the highest metal load, we expect to see a reduction in the ratio of carbon to nitrogen (C:N) in leaves, driven by the decreased development of cellulose and lignin and higher foliar metal concentration. We also expect to see accelerated decomposition in streams, associated with the reduced development of structural compounds and hence, faster physical fragmentation. Alternately, invertebrate richness, density and biomass associated with metal-enriched leaves could be lower due to more toxic environmental conditions and this could decrease the leaves' decomposition rate.

## **3. Materials and Methods**

### **3.1. Growth of Metal-Enriched Alder Trees**

We grew alder trees in metal-enriched soil to test the impact of BC's interim sediment quality guidelines on leaf quality and to produce alder leaves to use in further in-situ leaf pack experiments (Section 3.2). The interim sediment quality guidelines (ISQG) refer to environmental benchmarks for different water uses, including safe levels for aquatic life (BC MOE, 2006). The probable effect level (PEL) level refers to a level beyond which adverse effects may be seen (BC MOE, 2006). This experiment tested the effects of these guidelines for copper, cadmium and zinc aquatic soil concentrations on the growth and decomposition of alder leaves in stream ecosystems. To our knowledge, this is a novel approach to understanding the relationship between metal concentrations in riparian soil and subsequent in-stream decomposition of plants grown under those conditions.

#### **3.1.1. Alder Ecology**

Alder species are globally distributed in temperate, cool and alpine regions, while red alder (*Alnus rubra*) is found down the Pacific Northwest coast of North America (Pojar and MacKinnon, 2004; Roy et al., 2007). Red alder is widely distributed in moist environments, riparian zones and recently cleared land, as it is a fast growing colonizer species associated with early forest succession (Pojar and MacKinnon, 2004). Alder's early colonizing role and fast growth in nutrient-poor environments is due largely to its ability to form symbiotic associations with nitrogen-fixing actinomycete bacteria in their roots (Gaulke et al., 2006). While these associations may enrich depleted soil depleted in biologically-available nitrogen, they can also accelerate soil acidification and mobilization of trace metals (Mertens, 2004; Roy et al., 2007).

### 3.1.2. Greenhouse Growth Procedure

Twenty-five red alder saplings were purchased from Nat's Nursery on August 6<sup>th</sup>, 2009 and re-potted in their original soil plus a mixture of equal proportion greenhouse soil and peat mix and about 15% landscaping sand. They were stored outdoors on the Simon Fraser University campus until November 2009, when they were moved to a greenhouse. Trees were randomized to one of three treatments, including a control treatment (CTRL) with no metals applied. The other treatments were a mixture of copper, cadmium and zinc salts applied at British Columbia's interim sediment quality guidelines and probable effect level for these three metals (B.C. M.O.E., 2006). Plants were pruned to promote new leaf production in the spring. When buds started emerging in February 2010, the mixed metal solution was applied to the potting soil around each tree. We estimated that each pot contained 9.6kg dry sediment by sampling a known volume of sediment. This was used to calculate the metal dosage per pot (Table 1).

**Table 1. British Columbia's Interim sediment quality guidelines (ISQG) and Probable effect level (PEL) for cadmium, copper and zinc, and amount of metal salt added to potted alders (BC MOE, 2009).**

Metal	Metal Salt	ISQG (mg/kg)	ISQG dosage (g)	PEL (mg/kg)	PEL dosage (g)
Copper	CuCl <sub>2</sub> •2H <sub>2</sub> O	0.6	0.88	3.5	4.79
Cadmium	CdCl <sub>2</sub> •2½H <sub>2</sub> O	35.7	0.0064	197	0.012
Zinc	ZnCl <sub>2</sub>	123	2.20	315	5.4

Trees were dosed with metal mixtures on March 12<sup>th</sup>, 2010. They were watered every 2-3 days for three months until we manually harvested the leaves from the trees in July 2010. Leaves were air-dried in lab for two weeks, then dried at 60°C for a minimum of 48 hours before being randomly assigned to 5 g batches. Following dry-weighing, leaves were briefly wetted to soften them and prevent breakage as we sealed them in 15 cm x 15 cm, 10 mm plastic mesh bags.

### 3.1.3. Trace Metal Analysis

All metal analysis was performed using acid-washed apparatus. Leaves were digested for atomic absorption spectrophotometry analysis according to lab protocol (e.g. Bendell and Feng, 2009). Random sample of 10-20 leaves from each treatment were collected for metal analysis. Leaves were ground using a mortar and pestle, dried

at 60°C for at least 48 hours and weighed to  $0.15 \pm 0.01$  g. Leaves were heated with 10 ml environmental grade (70%) nitric acid in a flask on a hotplate at 110°C until 0.5 ml liquid remained. This was diluted to 10 ml with deionized water. Standard reference material (NBS tomato leaf) and blanks were digested and analyzed concurrently with leaf samples as quality controls. Samples were aspirated on a Perkin-Elmer AAnalyst 100 according to lab protocol.

### **3.1.4. Carbon and Nitrogen Analysis**

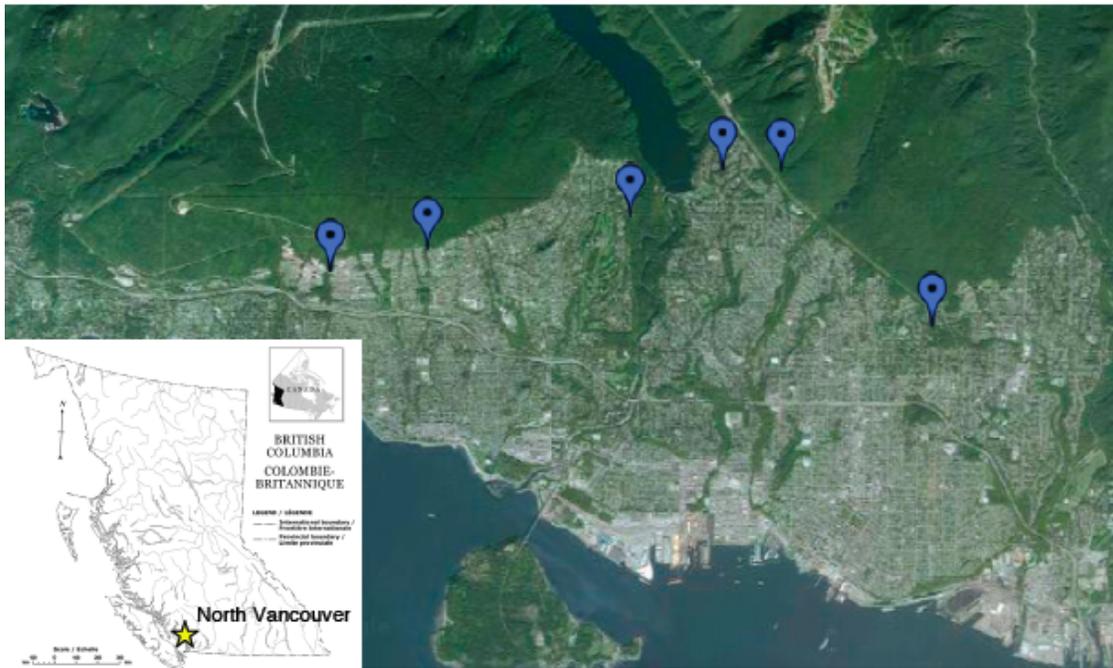
Sub-samples of leaf material were submitted for carbon and nitrogen content analysis at the University of British Columbia's Pacific Centre for Geochemical and Isotopic Research using a Vario EL cube (Elementar Americas Inc.). These values were used to calculate percent carbon and nitrogen as well as C:N ratio.

## **3.2. Field Study: Decomposition of Metal-Enriched Leaves**

### **3.2.1. Study Site**

We installed leaf packs in six small streams in North and West Vancouver, British Columbia. Streams were small tributaries located above most major urban development in these cities. Stream sites were chosen for similar sizes and North-South aspects, and accessibility at similar elevations to serve as replicates for the leaf pack experiments. Streams were dilute, slightly acidic, typically 2-4 m wide, shaded, forested streams, with alder present. Streams were accessed at 200-300m elevation above major urban development to minimize upstream metal contamination and disturbance. Grouse and Cypress Mountains were upstream of our sites, land used primarily for recreation. These land-use differences can be seen in satellite imagery below (Figure 1). From west to east, "MacDonald", a tributary to MacDonald Creek was accessed 10-15 m upstream of a large culvert under Chippendale Rd, West Vancouver. MacDonald Creek drains 3.74 km<sup>2</sup> over 6 km from Cypress Provincial Park, which is chanelized and culverted through West Vancouver to eventually drain at the Burrard Inlet (PSKF, 2009). The next site "Shields," a tributary to Lawson Creek was accessed from Shields Park, approximately 50m upstream from Pinecrest Dr. Lawson Creek drains 2.4 km<sup>2</sup> over 7.5 km, originating on Grouse Mountain, running through highly urbanized areas of West Vancouver,

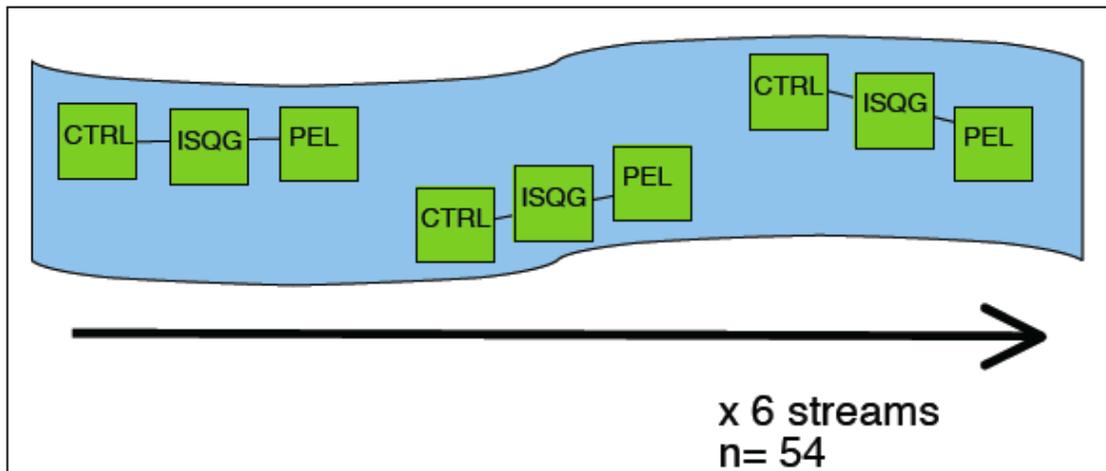
culverted several times before being channelized into Burrard Inlet (PSKF, 2009). The Shields Park site was the smallest stream at about 1 m wide. It was highly shaded with over-hanging vegetation. The “Capilano” site was a tributary to Houlgate Creek, accessed near Capilano Park. This stream was about 3 m wide. The “Grousewoods” and “MacKay” sites are tributaries to MacKay Creek, one of the largest and most threatened urban streams in North Vancouver, which drains 7.8 km<sup>2</sup> over 8.1 km through Grouse Mountain ski resort, residential, and industrial areas before discharging in the Burrard Inlet. MacKay Creek is highly impacted by urban development in North Vancouver. The tributary in Grousewoods Park was slow-moving, heavily-shaded, shallow, silty stream. The tributary “MacKay” accessed at Skyline Drive was a small, fast-moving headwater stream, accessed about 5-20 m upstream of a small bridge on a walking trail (PSKF, 2009). We accessed the “Princess” site, a tributary to Hastings Creek at Princess Park in North Vancouver. Unlike the other sites, there was some upstream residential development and culverting upstream. The stream was relatively wider than others at our sample point, approximately 5m wide. Hastings Creek, a tributary to Lynn Creek drains 7 km<sup>2</sup> over 23 km originating on Grouse Mountain and running through North Vancouver (PSKF, 2009). Like many other streams on the North Shore with important fish-bearing roles, this stream is also considered endangered because it is highly impacted by urban development (PSKF, 2009).



**Figure 1. Inset: North and West Vancouver, British Columbia. From West to East, the west tributary of MacDonal Creek accessed at 300 m elevation; a tributary to Larsen creek (Shields Park) at 370 m, a west tributary to Capilano Creek at 180 m, Grousewoods Park at 200 m (a tributary to MacKay Creek), a second tributary to MacKay Creek near Skyline Drive at 300 m, and Hastings Creek (Princess Park) at 240 m (Google Maps, 2012).**

### **3.2.2. Treatment Structure and Installation**

We installed nine leaf packs (three control, ISQG and PEL) in each of the six sites for a total  $n = 54$  leaf packs to be analyzed for decomposition and invertebrate assemblage. A fourth set of three leaf (one control, ISQG and PEL) packs was also installed per stream for carbon and nitrogen analysis of the leaves post-immersion. Within each treatment, leaf packs were randomly assigned to blocks and streams. To minimize the effects of spatial heterogeneity (Gjerløv and Richardson, 2004) within each 20-25 m stream sampling reach, we installed leaf packs in three blocks of one control, one ISQG and one PEL leaf pack from upstream to downstream. This also minimized the possibility that any metals would leach from the higher metal treatments into the ISQG or control leaves, respectively. We secured the packs to a nail using nylon string and then the streambed using rocks. Leaf packs were installed on October 18<sup>th</sup> and 20<sup>th</sup> and removed November 8<sup>th</sup> and 10<sup>th</sup>, 2010 (Figure 2).



**Figure 2. Leaf pack installation and treatment structure: control (CTRL), ISQG and PEL packs installed in groups to minimize effects of spatial heterogeneity within streams.**

### **3.2.3. Leaf Pack Removal and Processing**

Following three weeks' immersion, we removed the leaf packs and stored them in Ziplock™ bags. These were frozen within 5 hours for further processing. Leaf packs were defrosted one at a time into a white sorting tray. The mesh, and all debris clearly on the outside of the pack were discarded. We used a timed search effort of 30 minutes to pick invertebrates under a 5x magnification jeweller's lens. All alder fragments were then removed, dried for at least 72 hours at 60 °C, weighed and then burned for at least two hours at 550 °C to determine the ash-free dry mass (AFDM) remaining. Three randomly selected samples were searched until no additional invertebrates were found to ensure that packs were sufficiently searched to detect no new families. Invertebrates were later identified to family, with the exception of oligochaetes, identified only to subclass (Merritt, Cummins and Berg, 2008). Body length was also measured to the nearest 0.1 mm and dry biomass was estimated (Benke et al., 1999). Where family length-mass ratio was not presented, the most relevant genus was used to generate the estimate. Since oligochaetes were often damaged in preservation, length was estimated to be 10mm for all animals counted.

The fourth set of leaf packs was treated differently. Leaves were rinsed to remove any extraneous plant material or invertebrates, dried at 60 °C for at least 72 hours and homogenized for C:N analysis, methods as above. Because one set of leaves

was lost at Shields, this site was not included in C:N analysis at the end of the experiment.

### **3.2.4. Statistical Treatment and Analysis**

We used four response variables to describe the invertebrate communities: density, family richness, Simpson's diversity and total biomass. Statistical analysis was performed using JMP, Version 8. SAS Institute Inc., Cary, NC ©2010. Data were tested for normality using Shapiro-Wilk tests. If this test failed, non-parametric tests were used instead. Initial concentrations of copper, cadmium, zinc, were compared using Kruskal-Wallis approximation of a chi-squared test as data were not normal. Leaf size, carbon and nitrogen content were compared between treatments using one-way ANOVAs. Post-immersion, percent leaf mass remaining was calculated as ash-free dry mass divided by initial dry mass and compared between treatments using a two-way ANOVA, including metal treatment and stream site. Stream and treatment were considered fixed effects. Total invertebrate density, biomass and family-level richness were corrected for AFDM (g) leaf mass remaining. This correction was made because a greater leaf mass could theoretically support a larger number and biomass of invertebrates. Density per AFDM (g) remaining was log transformed to meet the assumption of normality and compared using a two-way ANOVA that accounted for metal treatment and site. The number of families per AFDM (g) were compared using a similar two-way ANOVA. Totally invertebrate biomass per gram of leaf remaining was not parametrically distributed and could not be transformed to meet the assumption, so site and metal treatment were compared with individual Kruskal-Wallis tests. Simpson's diversity was calculated using the USGS Invertebrate Data Analysis Software, *IDAS* (Cuffney, 2012), and compared between sites and treatments using Kruskal-Wallis tests. The density and biomass of common families (present in >75% of samples) were also compared using 2-way ANOVAs accounting for site and metal treatment.

## 4. Results

### 4.1. Alder Growth Experiment

Trees grown in soil treated to the probable effect level (PEL) for cadmium, copper and zinc were observed to have reduced leaf growth and browning (Figure 3), common signs of stress in alders (Roy et al., 2007).



**Figure 3. Healthy alder leaf from control treatment (left) and leaf from PEL treatment exhibiting possible symptoms of nutrient deficiency (right).**

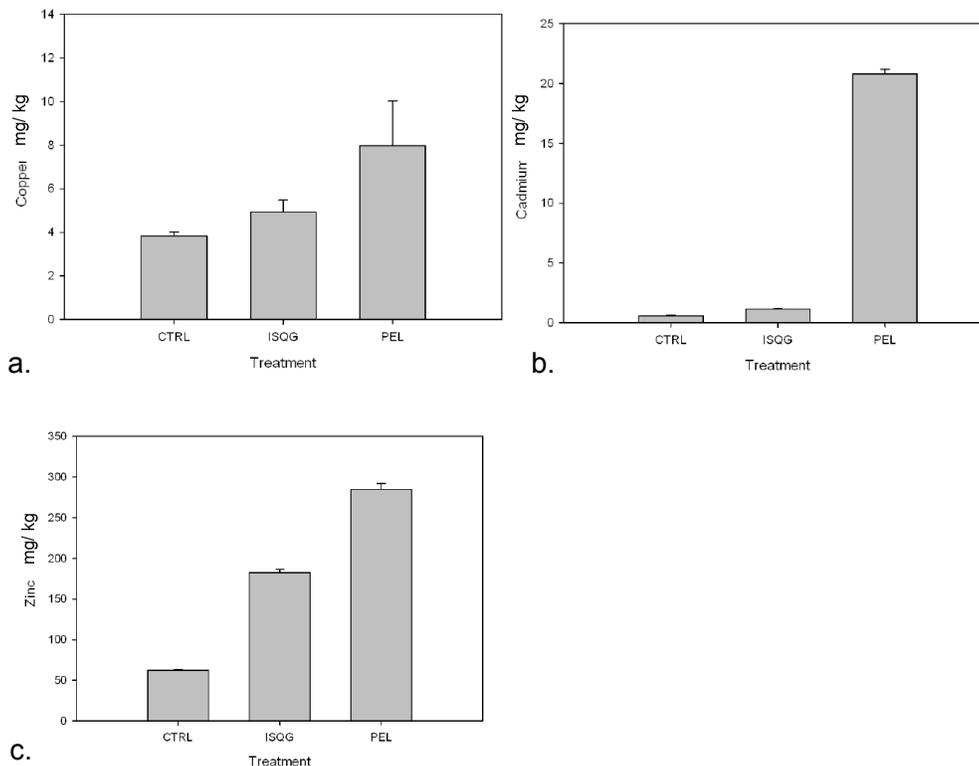
#### 4.1.1. Metal Uptake

Leaf concentration of copper, zinc and cadmium increased with metal treatment (Table 2). PEL treated leaves contained significantly more metal than other treatments (Tukey-HSD  $p < 0.05$ ; Figure 4a-c). Copper uptake increased with dosage (Kruskal-Wallis  $H_2 = 9.9$ ;  $p = 0.0071$ ;  $n = 16$ ), with PEL uptake significantly higher than the control or ISQG treatments (Tukey-Kramer HSD;  $q^* = 2.64$ ;  $\alpha = 0.05$ ). Cadmium uptake

increased with treatment (Kruskal-Wallis:  $H_2 = 7.2$ ;  $p = 0.03$ ;  $n = 9$ ). Cadmium uptake was significantly higher in the PEL treatment group than the control or ISQG groups (Tukey-Kramer HSD;  $q^* = 3.07$ ;  $\alpha = 0.05$ ). Zinc uptake varied between treatments (Kruskal-Wallis:  $H_2 = 7.2$ ;  $p = 0.03$ ;  $n = 9$ ), and was significantly different between all treatments (Tukey-Kramer HSD;  $q^* = 3.07$ ;  $\alpha = 0.05$ ).

**Table 2. Mean copper, cadmium and zinc concentration in leaves corresponding to metal treatments, including one standard error ( $\pm 1$  S.E.).**

Treatment	Copper $\pm 1$ S.E.	Cadmium $\pm 1$ S.E.	Zinc $\pm 1$ S.E.
CTRL	3.9 $\pm$ 0.1	0.58 $\pm$ 0.06	62 $\pm$ 1
ISQG	5.4 $\pm$ 0.4	1.2 $\pm$ 0.04	183 $\pm$ 4
PEL	10.9 $\pm$ 2.1	20.8 $\pm$ 0.4	285 $\pm$ 7



**Figure 4. a-c. Mean copper, cadmium and zinc uptake (mg/kg) in alder leaves prior to leaf pack construction ( $\pm 1$  S.E.).**

### 4.1.2. Leaf Quality

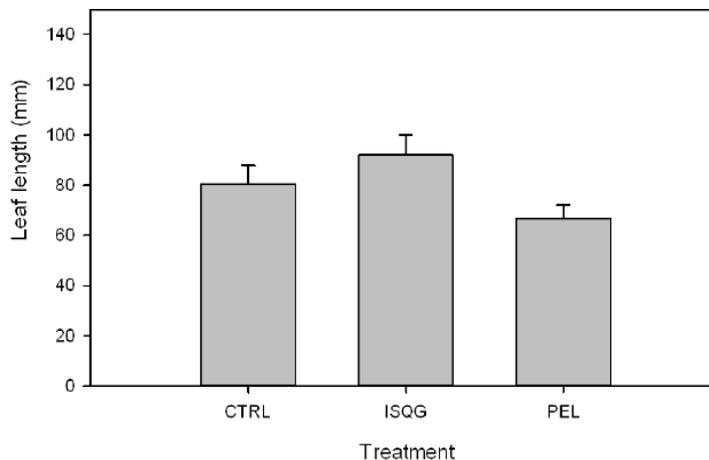
Leaf quality, as quantified by the ratio of carbon to nitrogen (C:N) differed between metal treatments ( $F_2 = 5.6$ ;  $p = 0.04$ ). This was driven by differences in carbon concentration ( $F_2 = 31.5$ ;  $p = 0.0007$ ), which were different between all metal treatments (Tukey-Kramer HSD;  $q^* = 3.7$ ;  $\alpha = 0.05$ ) rather than differences in nitrogen concentration, which were not significant ( $F_2 = 3.19$ ;  $p = 0.114$ ; Table 3).

**Table 3. Nitrogen and Carbon concentration of leaves ( $\pm 1$  S.E.)**

Treatment	n	%N $\pm 1$ S.E.	%C $\pm 1$ S.E.	C:N $\pm 1$ S.E.
CTRL	n=3	2.8 $\pm$ 0.1	46.9 $\pm$ 0.3	16.8 $\pm$ 0.5
ISQG	n=3	2.7 $\pm$ 0.1	45.9 $\pm$ 0.1	17.3 $\pm$ 0.5
PEL	n=3	3.0 $\pm$ 0.1	44.8 $\pm$ 0.1	15.0 $\pm$ 0.6

### 4.1.3. Leaf Size

Leaf length was significantly different between metal treatments (ANOVA:  $F_2 = 3.28$ ;  $p = 0.045$ ;  $n = 60$ ). Leaves from the PEL treatment were approximately 25 mm smaller than the ISQG treatment (Tukey-Kramer HSD:  $q^* = 2.4$ ;  $\alpha = 0.05$ ;  $p = 0.035$ ), but there were no other significant differences between treatments (Figure 5).



**Figure 5. Mean leaf length including one standard error ( $\pm 1$  S.E.).**

## **4.2. Field Study: Decomposition of Metal-Enriched Leaf Material**

### **4.2.1. Leaf Decomposition**

After immersion in study streams for 21 days, there was an average of 17% more mass lost from PEL than control treatment leaf packs. Percent leaf mass remaining was significantly different between treatments (2-way ANOVA:  $F_{2,5} = 15.5$ ;  $P < 0.0001$ ) and stream sites ( $F_{5,2} = 3.0$ ;  $P = 0.02$ ). Leaves grown under the highest metal treatment had the least mass remaining (Tukey-Kramer HSD;  $q^* = 2.41$ ,  $\alpha = 0.05$ ; Table 4). While % carbon in leaves post-immersion differed by site (2-way ANOVA;  $F_{4,2} = 5.48$ ;  $p = 0.013$ ), there was no significant difference between treatments (2-way ANOVA;  $F_{2,4} = 1.33$ ;  $p = 0.30$ ).

### **4.2.2. Invertebrate Community Structure**

#### **4.2.2.1. Density, Biomass and Univariate Community Indices**

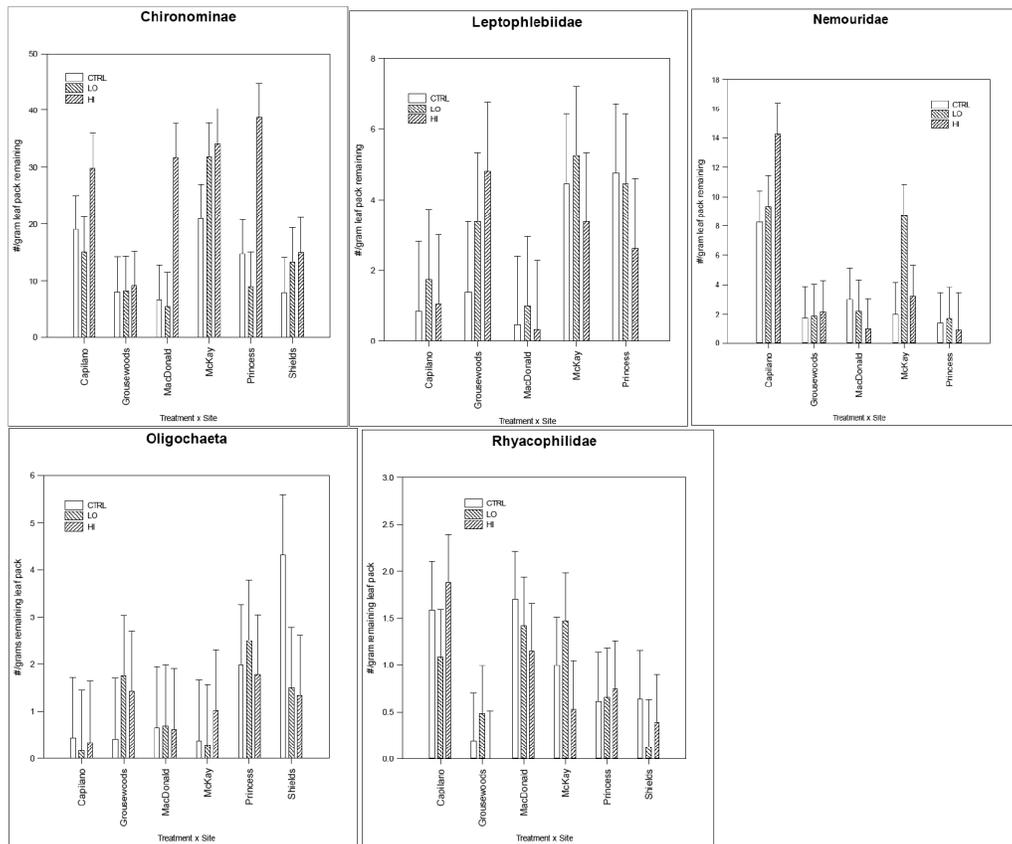
We compared density, family-level richness, and biomass per leaf mass remaining as well as Simpson's diversity to describe differences in invertebrate communities between leaf pack treatments (Table 4). Density and family richness per leaf mass remaining varied with site and treatment and was highest in the PEL treatment (2-way ANOVA;  $p < 0.05$ ). Simpson's diversity varied with site (Kruskal-Wallis:  $H_5 = 28.9$ ;  $p < 0.0001$ ), but was not significantly different between treatments (Kruskal-Wallis:  $H_2 = 3.06$ ;  $p = 0.22$ ). Total invertebrate biomass per leaf mass remaining did not vary with site (Kruskal-Wallis:  $H_5 = 6.2$ ;  $p = 0.29$ ) or treatment (Kruskal-Wallis:  $H_2 = 0.18$ ;  $p = 0.91$ ). Due to the overwhelming influence of *Pteronarcys* and oligochaetes in the total biomass, we investigated the biomass and density of common families separately to further elucidate any differences between leaf pack treatments.

**Table 4. Summary of average leaf mass remaining, invertebrate density, family richness, total invertebrate biomass and Simpson's Diversity Index by treatment, including standard error.**

Site	Treatment	Avg AFDM remaining (g) $\pm$ 1SE	Density/ AFDM (g) $\pm$ 1SE	Family richness/ AFDM (g) $\pm$ 1SE	Total biomass (mg) $\pm$ 1SE	Simpson's Diversity $\pm$ 1SE
Capilano	CTRL	2.28 $\pm$ 0.06	34.21 $\pm$ 7.19	3.81 $\pm$ 0.42	0.011 $\pm$ 0.0037	0.63 $\pm$ 0.03
	ISQG	2.12 $\pm$ 0.05	34.64 $\pm$ 5.50	4.43 $\pm$ 0.66	0.011 $\pm$ 0.0036	0.72 $\pm$ 0.02
	PEL	1.30 $\pm$ 0.31	58.64 $\pm$ 13.07	6.17 $\pm$ 0.73	0.035 $\pm$ 0.012	0.65 $\pm$ 0.04
Grouse-woods	CTRL	2.12 $\pm$ 0.31	19.56 $\pm$ 6.70	2.72 $\pm$ 0.76	0.89 $\pm$ 0.81	0.70 $\pm$ 0.06
	ISQG	1.96 $\pm$ 0.15	21.09 $\pm$ 1.61	4.51 $\pm$ 0.68	1.01 $\pm$ 0.99	0.78 $\pm$ 0.02
	PEL	1.52 $\pm$ 0.24	29.00 $\pm$ 5.90	5.37 $\pm$ 1.07	0.015 $\pm$ 0.0059	0.74 $\pm$ 0.02
MacDonald	CTRL	2.03 $\pm$ 0.33	17.69 $\pm$ 3.00	3.24 $\pm$ 0.27	0.0083 $\pm$ 0.0011	0.71 $\pm$ 0.12
	ISQG	1.88 $\pm$ 0.11	17.44 $\pm$ 5.32	4.03 $\pm$ 0.70	0.0099 $\pm$ 0.0053	0.75 $\pm$ 0.04
	PEL	0.63 $\pm$ 0.32	93.79 $\pm$ 69.02	41.65 $\pm$ 36.98	0.0093 $\pm$ 0.0049	0.47 $\pm$ 0.02
MacKay	CTRL	2.19 $\pm$ 0.42	31.03 $\pm$ 1.03	3.26 $\pm$ 0.61	0.0088 $\pm$ 0.0030	0.46 $\pm$ 0.13
	ISQG	1.77 $\pm$ 0.50	48.63 $\pm$ 6.32	3.78 $\pm$ 1.38	0.0089 $\pm$ 0.0011	0.46 $\pm$ 0.14
	PEL	1.88 $\pm$ 0.05	43.87 $\pm$ 10.01	3.00 $\pm$ 0.40	0.011 $\pm$ 0.0087	0.33 $\pm$ 0.12
Princess	CTRL	2.18 $\pm$ 0.22	31.50 $\pm$ 8.88	2.82 $\pm$ 0.41	0.021 $\pm$ 0.0074	0.67 $\pm$ 0.01
	ISQG	2.09 $\pm$ 0.13	23.64 $\pm$ 4.91	3.52 $\pm$ 0.12	0.029 $\pm$ 0.021	0.57 $\pm$ 0.12
	PEL	1.29 $\pm$ 0.55	48.77 $\pm$ 12.64	5.16 $\pm$ 2.00	0.031 $\pm$ 0.019	0.35 $\pm$ 0.16
Shields	CTRL	2.68 $\pm$ 0.19	13.06 $\pm$ 0.56	0.64 $\pm$ 0.16	0.042 $\pm$ 0.035	0.12 $\pm$ 0.07
	ISQG	2.63 $\pm$ 0.07	15.04 $\pm$ 1.03	1.01 $\pm$ 0.24	0.013 $\pm$ 0.0036	0.20 $\pm$ 0.06
	PEL	1.83 $\pm$ 0.24	18.67 $\pm$ 5.18	2.09 $\pm$ 0.46	0.013 $\pm$ 0.0037	0.31 $\pm$ 0.05
Total	CTRL	2.25 $\pm$ 0.11	24.51 $\pm$ 2.73	2.74 $\pm$ 0.29	0.16 $\pm$ 0.14	0.55 $\pm$ 0.06
	ISQG	2.08 $\pm$ 0.10	26.75 $\pm$ 3.22	3.54 $\pm$ 0.39	0.18 $\pm$ 0.17	0.58 $\pm$ 0.06
	PEL	1.41 $\pm$ 0.15	48.79 $\pm$ 11.70	10.57 $\pm$ 6.19	0.019 $\pm$ 0.004	0.48 $\pm$ 0.05

#### 4.2.2.2. Abundance of Common Taxa

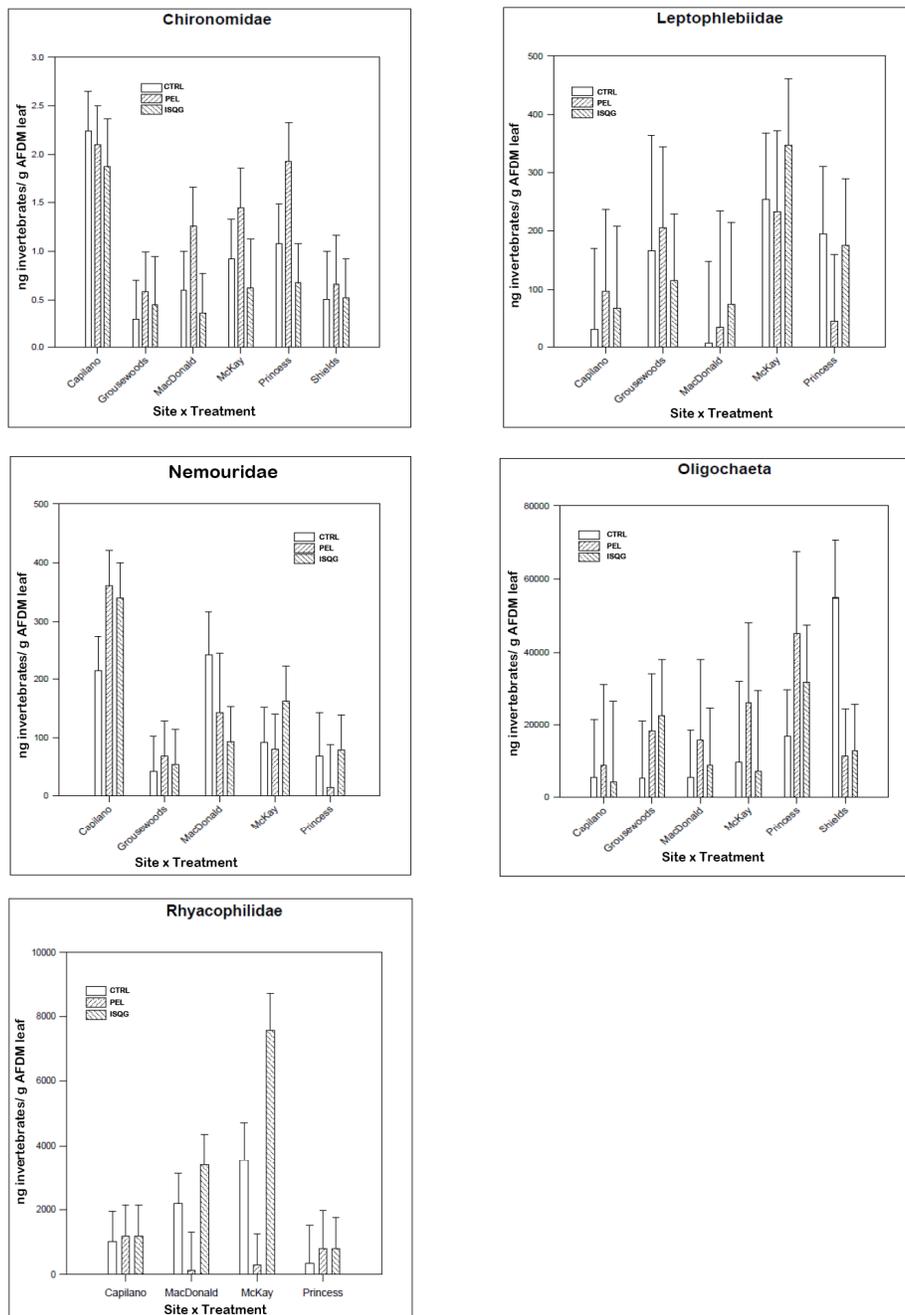
We decided to investigate the abundance and biomass of taxa that occurred in more than 75% of samples, controlled for leaf mass (AFDM) remaining. Full abundance data are available in Appendix A. After establishing that Chironomidae, Leptophlebiidae, Nemouridae, Rhyacophilidae and Oligochaeta as the five most common taxa, we used a series of 2-way ANOVAs (ranked data if non-normal) to determine whether site or metal treatment impacted the density of invertebrates in the leaf pack. Chironomid density differed with site (2-way ANOVA on ranks;  $F_{5,2} = 4.4$ ;  $p = 0.003$ ) and treatment (2-way ANOVA on ranks;  $F_{2,5} = 9.2$ ;  $p < 0.001$ ). The density of chironomids was higher in PEL leaf packs than CTRL or ISQG (Holm-Sidak method;  $p < 0.05$ ) and MacKay was different than Grousewoods and Shields (Holm-Sidak method;  $p < 0.05$ ). Leptophlebiidae density did not differ by site or treatment (2-way ANOVA;  $p > 0.05$ ). Nemouridae density differed with site (2-way ANOVA on ranks;  $F_{5,2} = 7.8$ ;  $p < 0.001$ ), but not treatment. Nemouridae density was significantly higher at Capilano than at Princess, MacDonald or Grousewoods (Holm-Sidak method;  $p < 0.05$ ). Oligochaeta density did not differ significantly with treatment or site (2-way ANOVA on ranks;  $p > 0.05$ ). Rhyacophilidae density differed with site (2-way ANOVA on ranks;  $F_{5,2} = 3.3$ ;  $p < 0.015$ ), but not treatment.



**Figure 6. Density of common taxa on leaf packs: Chironomidae, Leptophlebiidae, Nemouridae, Oligochaeta and Rhyacophilidae ( $\pm 1$  S.E.).**

#### 4.2.2.3. Biomass of Common Taxa

As above, we tested whether site and treatment impacted the estimated biomass of common taxa Chironomidae, Leptophlebiidae, Nemouridae, Rhyacophilidae and Oligochaeta within leaf packs. Only Nemouridae (two-way ANOVA;  $F_{5,2} = 8.5$ ;  $p < 0.001$ ) and Chironomidae (two-way ANOVA;  $F_{5,2} = 5.6$ ;  $p < 0.001$ ) differed significantly with site. No other taxa showed a significant relationship between biomass and treatment or site (Figure 7). Data available in Appendix B.



**Figure 7. Mean total biomass per treatment by site with for the five most common families or subclass ( $\pm 1$  S.E.). From left-top clockwise: Chironomidae, Leptophlebiidae, Oligochaeta, Rhyacophilidae, Nemouridae). Note: All families were present at all sites, with the exception of Leptophlebiidae at Shields, the site with the lowest invertebrate recovery. Families that were not represented in all three treatments were not included in the figure.**

## 5. Discussion

We hypothesized that alders grown under metal enriched conditions would uptake more metals, display reduced leaf quality (reduced carbon to nitrogen ratio), and decompose faster in streams. These hypotheses were all supported in our findings. We further predicted that invertebrate communities associated with metal-enriched leaf packs would be characterized by lower density, biomass and taxonomic richness, which our results did not support.

### 5.1. Leaf Quality

We found a significant difference in zinc and cadmium between all treatments and copper uptake between the PEL metal treatment and the ISQG and control treatments. We hypothesized that zinc would increase with metal dosage, and uptake was significantly higher in the PEL treatment group than the control or ISQG groups. These concentrations are consistent with Baltreñaite and Butkus' (2007) findings that zinc tended to accumulate in higher quantities away from the roots with leaf concentrations ranging from 141 to 271 mg/ kg in black alder. Our results are additionally consistent with Gaulke et al. (2006) who found that foliar zinc concentrations increased with and closely resembled metal-rich biosludge amendments, resulting in leaf concentrations between 249-279 mg/ kg in red alder. While it was expected that PEL leaves would have the highest copper load of all three treatments, there was no significant difference in copper concentration between the control and ISQG treatments. This is consistent with Baltreñaite and Butkus' (2007) simulation of copper uptake in black alder, which found leaf concentrations from 2.5 to 11.5 mg/ kg, with greater accumulation occurring in the stem than leaves. Furthermore, Nikula et al. (2010) found that land use type did not have an impact on copper uptake between rural and urban environments, unlike zinc and cadmium. As predicted, cadmium concentration in leaves

increased with metal dosage and was significantly different between treatments, consistent with the findings of Wickliff et al. (1980). Cadmium is a toxic, non-essential metal that can cause nutrient deficiency in plants by utilizing other micronutrient uptake pathways, such as those of zinc and copper to accumulate in above-ground tissues (Redjala et al., 2009). The sharply increased uptake of cadmium in the ISQG and PEL treatments could also be preventing some of the uptake of copper and zinc, and could explain why we did not see increased copper uptake (Redjala et al., 2009).

Leaf quality, as measured by the ratio of carbon to nitrogen (C:N) differed between metal treatments was driven by differences in carbon concentration rather than nitrogen, which was not significant. Carbon concentration was 2% less in the PEL treatment than the control, resulting in a C:N ratio of 17 in the control and ISQG treatments, but only 15 in the PEL treatment. These findings are in contrast to Nikula et al. (2010)'s findings that differences in C:N in poplars from urban and rural areas were driven primarily by differences in nitrogen. Here we find that carbon drives differences in the C:N ratio and the lower carbon uptake in the PEL leaves actually results in a lower C:N ratio, which is the opposite trend to what we predicted. The reduction in carbon could be a result of reduced development of structural compounds like lignin. Likewise, Nikula et al. also found a reduction in lignin and phenolics in leaves from urban areas. Cadmium may additionally interfere with auxin development and therefore, metabolism and lignin production, as has been demonstrated in poplars (Elobeid et al., 2012). Cadmium interferes with nodulation initiation in seedlings and availability of nutrients, and thus, the development of structural compounds (Wickliff et al., 1980). Leaves grown under the highest metal treatment were significantly smaller as well, which is consistent with Nikula et al.'s (2010) finding of lower specific leaf area and faster decomposition of urban leaves exposed to higher metal levels than rural leaves. Additionally, alder's association with nitrogen-fixing bacteria promotes soil acidification, a consequence of which could be increased trace metal mobilization (Roy et al., 2007). Thus, differences in carbon concentrations of leaves could be more pronounced in alders grown in metal-contaminated environments.

## 5.2. Leaf Decomposition

Leaves grown under the highest metal treatment decomposed the fastest in-stream and had the lowest percentage of AFDM remaining. This study demonstrated a linkage between metal-enrichment and reduced C:N of leaves grown under the PEL metal treatments. Thus, reduced structural integrity and smaller leaf length could have contributed to faster physical fragmentation of leaves in stream. Leaf decomposition is mediated by internal leaf qualities such as species and nutrient quality, as well as environmental factors—from abiotic properties of the water such as chemistry, turbidity, thermal and hydrographical regimes to biotic factors such as microbial and invertebrate decomposer assemblages, which may have further unique interactions with the environment (Chadwick et al., 2007). Urbanization may additionally exacerbate several of these factors given the functional complexity of the decomposition process (Chadwick et al., 2007; Paul et al., 2006; Walsh et al., 2005). We tried to mitigate these factors by choosing streams that were upstream of major urban developments, however, some were accessed from parks, and may have been affected by land management such as the clearing of plant material to build roads and paths. While the majority of land upstream of our sites was forested parkland, localized disturbance may have created higher storm flashes and more sedimentation than would be found in natural, undisturbed streams. These factors can accelerate physical fragmentation of leaves. Overall, we tried to minimize exposure of our leaf packs to upstream disturbance so that abiotic factors would not interfere with the biological leaf breakdown process.

While metals have well-documented toxicological impacts on stream invertebrate and microbial communities, there are many examples of functional resilience in impacted systems (Clements et al., 2010; Carlisle and Clements, 2005, Woodcock and Huyrn, 2004). Even along gradients of high metal pollution, there may be serial replacement of functionally-important species, which can mitigate the overall impact on functional processes (Schmera et al., 2012; Woodcok and Huyrn, 2004). Conversely, Powers and Salute (2011) found that Zn enrichment actually increased leaf decomposition, following immersion in zinc solution. Additional microcosm experiments have shown that once leaf disks are released from the stress of copper and zinc immersion, fungal communities can recover quickly, as can their ability to process organic material (Duarte et al., 2009).

### 5.3. Invertebrate Community Metrics

As hypothesized, family-level richness and density, when corrected for AFDM were statistically different between sites and treatments. Density was generally higher in the PEL metal treated leaves, which could be a function of resident invertebrate communities persisting on decreasing volumes of leaves. Conditioned leaves in advanced stages of decomposition can provide richer food resources to collector-gatherers, scrapers and shredders, resulting in higher invertebrate densities, particularly during intermediate stages of leaf decomposition (Tanaka et al., 2006). While there is extensive evidence that invertebrate richness can be reduced by metal contamination (Poulton et al., 2010; Carlisle and Clements, 2005), richness is also affected by litter quality, nutrient availability and fungal conditioning of leaves, which increases nutrient concentration (Woodcock and Huyrn, 2004; Graça, 2001). Differences in richness detected by this study may be related to differences in fungal conditioning status, rather than metal concentration in leaves. In contrast, invertebrate density has also been shown to increase with time, as litter becomes more palatable to shredding species (Alonso et al., 2010). In this study, given that most of the PEL leaves were collected after >50% mass loss, it is possible that the remaining material contained a higher percent recalcitrant material and therefore was less palatable to shredding invertebrates (Muto et al., 2011). Robinson and Joliden (2005) found no clear temporal trend in richness or density on leaf packs immersed in different stream types. Additionally, Sylvestre and Bailey (2005) found no relationship between leaf pack invertebrate community and decomposition stage or organic carbon. Additionally, the ability to detect community differences in leaf pack studies is affected by temporal resolution and the stage in the trajectory of decomposition that leaf packs are sampled, which will be inherently difficult to standardize if there are significant differences in decomposition rate between treatment (Mancinelli et al., 2007). Total invertebrate biomass per leaf mass remaining did not show any relationship with site or treatment. Similarly, Woodcock and Huyrn (2004) did not find any differences in total biomass per leaf pack along a pollution gradient, although the percent biomass contributed by different functional groups did change. At the family level, our study only found that *Chironomidae* biomass and *Nemouridae* biomass showed a relationship with site and none of the common families showed a relationship with metal treatment.

Given differences in density and richness in both sites and treatments, we further investigated whether these trends were reflected in the five most common taxa. While none of the biomass of the five common families showed a relationship with metal treatment, *Chironomidae* density on PEL leaves was higher than on ISQG or control leaf packs. Since PEL leaf packs were typically further decomposed, this finding is potentially due to chironomids' common role as collector-gatherers, which may be facilitated by previous conditioning and breakdown of the leaves (Sylvestre and Bailey, 2005). Additionally, Nemouridae density was much higher at the Capilano tributary than all other streams (excluding MacKay). The sampling site at Capilano appeared to be less disturbed than other sites, with a higher degree of structural complexity. Generally, sites were as controlled as possible for similarity, but as to also be reflective of streams that could be subjected to increased urban pressures in Vancouver, BC.

Further resolution to genus or species may have allowed us to elucidate functional roles more accurately, however, given considerations of time and funding allocation, this was decided against. Chironomids also had very low biomass, and relatively wide size range thus biomass could be easily biased by the presence of one or two larger individuals. Chironomids may have also been subject to more visual bias in the search process, given that the size range of chironomids goes below visual detection. While further resolving our invertebrate communities to genus could have been helpful, considerable research suggests a high correlation between family-level and genus-species level identification particularly in detecting impact (Bowman and Bailey, 1997). However, detecting functional impacts may be more difficult than describing a community structure (Bowman and Bailey, 1997). Taxonomic sufficiency depends on the magnitude of impact, as well as the spatial scale in which to detect community differences (Mandelik et al. 2007). Medeiros et al. (2010) examine taxonomic sufficiency in detecting differences in macroinvertebrate assemblages from metal-impacted Arctic streams. While family-level identification was sufficient to detect differences between pristine and impacted streams, tribe and sub-family level identification had more power to detect differences in diversity on an impact gradient (Medeiros et al. 2010). Buss and Vitorino (2010) found family and genus-level identification were similarly effective at detecting differences in ecological status of

streams but family was more cost-effective. Such considerations were part of our decision to evaluate invertebrates to family only.

## 6. Conclusion

Here, we present a new approach to understanding the highly complex effects of metal pollution on riparian and stream ecosystems. We provide further evidence that *Alnus rubra* uptake metals in response to environmental availability and their foliar concentrations are consistent with that of the literature. Given the well-established relationship between the reduced development of structural compounds in leaves and metal pollution, we show that leaves exposed to metal-enrichment have a faster decomposition trajectory in urban streams than those grown under lower metal conditions. Additionally, richness and density of invertebrates associated with these leaves was increased on the higher metal leaves, potentially a result of an advanced decomposition state, and resident invertebrates on a more rapidly decreasing amount of leaf material. If the processing of leaf inputs from impacted riparian zones is accelerated in-stream, it could limit downstream availability of particulate organic material. Given the complex nature of functional processes in stream ecosystems, the effects of metal-contamination on organic matter processing warrants further study. Future studies should consider the effects of catchment-wide metal pollution, other common riparian plants as well as different metal conditions.

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## **Appendices**

## Appendix A.

### Abundance of common taxa by site

#### 1. Capilano

Lowest taxon identified	Control	ISQG	PEL
Baetidae	0	6	4
Chironomidae	131	96	109
Chloroperlidae	1	4	2
Elmidae	3	2	4
Gammaridae	0	0	0
Heptageniidae	14	13	16
Isopoda	0	0	0
Lepidostomatidae	2	1	2
Leptophlebiidae	6	11	5
Leucridae	0	0	0
Limnephilidae	1	3	1
Nemouridae	57	59	47
Oligochaeta	3	1	2
Pteronarcyidae	0	0	0
Rhyacophilidae	11	7	7
Simuliidae	3	15	9
Tipulidae	1	1	0

## 2. Grousewoods

Lowest taxon identified	Control	ISQG	PEL
Baetidae	1	4	1
Chironomidae	48	48	35
Chloroperlidae	0	0	1
Elmidae	2	4	5
Gammaridae	0	0	0
Heptageniidae	1	1	0
Isopoda	0	0	0
Lepidostomatidae	1	2	0
Leptophlebiidae	8	20	24
Leucridae	0	0	1
Limnephilidae	0	2	5
Nemouridae	10	12	10
Oligochaeta	3	11	6
Pteronarcyidae	2	1	0
Rhyacophilidae	1	3	0
Simuliidae	38	15	34
Tipulidae	0	1	3

## 3. MacDonald

Lowest taxon identified	Control	ISQG	PEL
Baetidae	0	1	0
Chironomidae	34	30	38
Chloroperlidae	11	28	4
Elmidae	0	0	0
Gammaridae	0	0	0
Heptageniidae	0	1	0
Isopoda	0	2	0
Lepidostomatidae	15	2	0
Leptophlebiidae	3	6	1
Leucridae	0	0	0
Limnephilidae	9	2	0
Nemouridae	20	13	3
Oligochaeta	4	4	2
Pteronarcyidae	0	0	0
Rhyacophilidae	8	8	3
Simuliidae	0	2	3
Tipulidae	0	0	0

#### 4. McKay

<b>Lowest taxon identified</b>	<b>Control</b>	<b>ISQG</b>	<b>PEL</b>
Baetidae	2	1	0
Chironomidae	147	177	195
Chloroperlidae	2	3	5
Elmidae	5	0	0
Gammaridae	0	0	0
Heptageniidae	0	0	0
Isopoda	0	0	0
Lepidostomatidae	6	0	2
Leptophlebiidae	21	20	19
Leucridae	0	0	0
Limnephilidae	0	0	0
Nemouridae	13	34	18
Oligochaeta	3	2	6
Pteronarcyidae	0	0	0
Rhyacophilidae	5	6	3
Simuliidae	0	1	0
Tipulidae	0	1	2

#### 5. Princess

<b>Lowest taxon identified</b>	<b>Control</b>	<b>ISQG</b>	<b>PEL</b>
Baetidae	0	0	1
Chironomidae	95	77	102
Chloroperlidae	2	3	5
Elmidae	5	0	0
Gammaridae	0	0	0
Heptageniidae	0	0	0
Isopoda	0	0	0
Lepidostomatidae	6	0	2
Leptophlebiidae	21	20	19
Leucridae	0	0	0
Limnephilidae	0	0	0
Nemouridae	13	34	18
Oligochaeta	3	2	6
Pteronarcyidae	0	0	0
Rhyacophilidae	5	6	3
Simuliidae	0	1	0
Tipulidae	0	1	2

## 6. Shields

<b>Lowest taxon identified</b>	<b>Control</b>	<b>ISQG</b>	<b>PEL</b>
Baetidae	0	0	0
Chironomidae	69	105	73
Chloroperlidae	0	0	0
Elmidae	0	0	0
Gammaridae	0	0	0
Heptageniidae	0	0	0
Isopoda	0	0	1
Lepidostomatidae	0	0	0
Leptophlebiidae	0	0	0
Leucridae	0	0	0
Limnephilidae	0	0	0
Nemouridae	0	0	3
Oligochaeta	39	12	8
Pteronarcyidae	0	0	0
Rhyacophilidae	5	1	2
Simuliidae	0	0	6
Tipulidae	2	1	0

## Appendix B.

### Biomass of common taxa by site

#### 1. Capilano

Lowest taxon identified	Control (µg)	ISQG (µg)	PEL (µg)
Baetidae	0	0.24	0.28
Chironomidae	0.016	0.050	0.0085
Chloroperlidae	0.012	0.12	0.046
Elmidae	9.47	4.93	14.75
Gammaridae	0	0	0
Heptageniidae	29.16	47.24	96.68
Isopoda	0	0	0
Lepidostomatidae	0.44	0.019	0
Leptophlebiidae	0.14	0.29	0.27
Leucridae	0	0	0
Limnephilidae	0.0076	0.15	0.0092
Nemouridae	1.48	2.13	1.25
Oligochaeta	25.48	8.49	16.98
Pteronarcyidae	0	0	0
Rhyacophilidae	5.36	7.58	4.85
Simuliidae	0.036	1.158	0.27
Tipulidae	3.25	0.15	0

## 2. Grousewoods

Lowest taxon identified	Control (µg)	ISQG (µg)	PEL (µg)
Baetidae	0	0.12	0.019
Chironomidae	0.0017	0.045	0.0024
Chloroperlidae	0	0	0.14
Elmidae	1.09	15.39	14.03
Gammaridae	0	0	0
Heptageniidae	0.28	0.023	0
Isopoda	0	0	0
Lepidostomatidae	0.22	0.22	0
Leptophlebiidae	0.32	0.69	0.66
Leucridae	0	0	0.0013
Limnephilidae	0	1.43	0.086
Nemouridae	0.23	0.34	0.33
Oligochaeta	25.48	93.42	50.95
Pteronarcyidae	5631.85	5828.24	0
Rhyacophilidae	1.92	1.10	0
Simuliidae	2.50	0.81	2.59
Tipulidae	0	0.030	0.89

## 3. MacDonald

Lowest taxon identified	Control (µg)	ISQG (µg)	PEL (µg)
Baetidae	0	0.043	0
Chironomidae	0.0034	0.0020	0.0017
Chloroperlidae	0.49	1.33	0.17
Elmidae	0	0	0
Gammaridae	0	0	0
Heptageniidae	0	0.92	0
Isopoda	0	0.025	0
Lepidostomatidae	1.65	0.15	0
Leptophlebiidae	0.034	0.30	0.039
Leucridae	0	0	0
Limnephilidae	1.74	0.18	0
Nemouridae	1.07	0.56	0.15
Oligochaeta	33.97	33.97	16.98
Pteronarcyidae	0	0	0
Rhyacophilidae	11.95	18.62	0.23
Simuliidae	0	0.025	0.057
Tipulidae	0	0	0

#### 4. McKay

Lowest taxon identified	Control (µg)	ISQG (µg)	PEL (µg)
Baetidae	0.12	0.18	0
Chironomidae	0.0065	0.0086	0.0084
Chloroperlidae	0.071	0.044	0.21
Elmidae	19.50	0	0
Gammaridae	0	0	0
Heptageniidae	0	0	0
Isopoda	0	0	0
Lepidostomatidae	0.49	0	0.64
Leptophlebiidae	1.09	1.34	0.87
Leucridae	0	0	0
Limnephilidae	0	0	0
Nemouridae	0.58	0.70	0.46
Oligochaeta	25.48	16.98	50.95
Pteronarcyidae	0	0	0
Rhyacophilidae	10.18	20.18	1.71
Simuliidae	0	0.11	0
Tipulidae	0	7.94	7.48

#### 5. Princess

Lowest taxon identified	Control (µg)	ISQG (µg)	PEL (µg)
Baetidae	0	0	0.043
Chironomidae	0.0070	0.0042	0.0057
Chloroperlidae	0	0	0
Elmidae	0	6.61	0
Gammaridae	1.04	0.23	0
Heptageniidae	13.63	20.22	23.72
Isopoda	0.0059	0.0084	0
Lepidostomatidae	0	0	0
Leptophlebiidae	1.19	1.19	0.19
Leucridae	0	0	0
Limnephilidae	0	0	0
Nemouridae	0.29	0.52	0.046
Oligochaeta	118.89	144.37	93.42
Pteronarcyidae	0	0	0
Rhyacophilidae	1.29	5.31	3.06
Simuliidae	2.92	0.33	1.47
Tipulidae	0	0	0

## 6. Shields

<b>Lowest taxon identified</b>	<b>Control (µg)</b>	<b>ISQG (µg)</b>	<b>PEL (µg)</b>
Baetidae	0	0	0
Chironomidae	0.0025	0.0041	0.0029
Chloroperlidae	0	0	0
Elmidae	0	0	0
Gammaridae	0	0	0
Heptageniidae	0	0	0
Isopoda	0	0	0.0031
Lepidostomatidae	0	0	0
Leptophlebiidae	0	0	0
Leucridae	0	0	0
Limnephilidae	0	0	0
Nemouridae	0	0	0.0023
Oligochaeta	331.21	101.91	67.94
Pteronarcyidae	0	0	0
Rhyacophilidae	4.28	0.58	0.80
Simuliidae	0	0	0.12
Tipulidae	0.033	0.0026	0