METAL CONTAMINATION IN STREAMS IN THREE NEW ZEALAND CITIES, THE EFFECTS ON BENTHIC COMMUNITIES AND THE ACCUMULATION IN A NEW ZEALAND MAYFLY

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Abstract

Increasing urbanisation places pressure on the ecological integrity of streams in many towns and cities. Stormwater running over impervious surfaces carry contaminants into the receiving waterways and heavy metals are one of the main contaminants in stormwater. They are of concern because of their ubiquitness, toxicity, and persistance in the environment. There is limited literature in New Zealand on the metal contamination in urban streams and comparing different regions. Councils undertake monitoring programmes of their respective urban streams, however the monitoring and reporting process differs between urban centres.

This study investigated the metal contamination and effects on the benthic invertebrate community in three major urban centres in New Zealand; Auckland, Christchurch, and Wellington. Dissolved copper was the only metal (out of both the sediment and water) to be significantly different between cities, with concentrations in Christchurch being lower. Generally, copper (Cu), lead (Pb), and zinc (Zn) were the most common metals to exceed ANZECC guidelines. Chromium (Cr) and cadmium (Cd) were also identified as metals to potentially be of concern in the future. Sediment metal concentration largely increased together, however, significant relationships between water and sediment concentrations were not common. A smaller sediment size fraction of < 63µm had significantly higher concentrations for arsenic (As), Cu, and Zn.

The benthic invertebrate communities found in this study were similar to those found in literature, where diversity is decreased and pollution tolerant taxa are common. The Wellington benthic invertebrate communities were significantly different to both Auckland and Christchurch, exemplified by an NMDS and biotic indices. The biotic indices indicated better stream health for Wellington. A sediment metal index created from a principal component analysis was found to be a common predictor for the generalised linear models of the biotic indices, both for the whole dataset and the individual cities. However, the contribution of the sediment metal index was relatively low.

This study also investigated the accumulation of Cu and Zn, both as single metal species and as a mixture, by the *Deleatidium* spp. mayfly. Exposures were performed for both spiked water and spiked biofilm, to compare accumulation through water and diet. Concentrations of Cu in the water and for contaminating biofilm were 0, 1.4, 5, 80, 300, and 1000 µg L^{-1}. Mayfly Cu concentrations significantly increased at the 80 µg L^{-1} treatment in both the water and biofilm exposure. The biofilm exposure significantly increased at the higher treatments, however, the water exposure did not. Concentrations of Zn treatments were 0, 8,

50, 150, 1500, and 5000 μg L^{-1}. There were no significant changes in Zn accumulation in the mayflies for either the biofilm or water exposures. The Cu and Zn mixture produced the same results as the single metal species experiments. This indicates that Zn may be better regulated than Cu and that diet may be more important in accumulation of Cu.

The results of this thesis inidcate that metal contamination is relatively consistent across different urban areas in New Zealand and that sediment metal concentrations may be more useful for monitoring than water concentrations. The exposures suggest that studies should continue to investigate the accumulation of different metals through diet and water exposures, as Cu and Zn showed contrasting results.

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Abbreviations

ARC	Auckland Regional Council
ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
ANZECC	Australia and New Zealand Environment and Conservation Council
CBD	Central Business District
CCA	Canonical Correspondence Analysis
CF	Contamination Factor
DO	Dissolved Oxygen
EPA	Environmental Protection Authority
EPT	Ephemeroptera Plecoptera Trichoptera
FENZ	Freshwater Ecosystems of New Zealand
GLM	Generalised Linear Models
MCI	Macroinvertebrate Community Index
NMDS	Non-metric Multidimensional Scaling
%OM	percent Organic Matter
PCA	Principal Component Analysis
PLI	Pollution Load Index
RI	Risk Index
UCI	Urban Community Index

1 Chapter 1: Introduction

The conversion of Earth's land surface to urban use is one of the most significant human impacts on the environment (Seto *et al.* 2011). In 1950, 30% of the world's population lived in urban areas, by 2014 it had reached 54%, and the proportion is predicted to reach 66% by 2050 (United Nations 2014). This figure is much higher in the developed world with 85% predicted to be living in urban areas by 2050 (United Nations 2014). New Zealand has 70% of its population recorded as living in an urban area in 2001 (Statistics New Zealand. 2001). Increasing urbanisation raises questions regarding the negative impacts on the natural environment and possible mitigation strategies. This change in demography has and continues to bring about landscape transformations with documented and anecdotal effects on ecosystems (Paul and Meyer 2008; Kaushal and Belt 2012). While urban areas only cover a small percentage of Earth's land surface (only 2%), the ecological footprint is substantial (Folke *et al.* 1997). As a result, the world faces increasing numerous challenges to protect the environment in these urban areas.

One of the major challenges is protection of freshwater. This has become one of the key issues for the 21st century, both in terms of quantity and quality. In the past, drainage issues caused by decreases in permeable surfaces in towns and cities were solved by directing excess rainfall into nearby streams, essentially treating streams as drains, with them often being modified and concreted to accommodate the flows (Walsh *et al.* 2005). This caused significant damage to ecosystems in river networks within urban areas and is why urbanisation is often associated with loss of aquatic biodiversity (Allan 2004; Paul and Meyer 2008).

People tend to describe cities as places characterised by large human population densities (Rees 2003). Cities are rarely described in terms of their ecological function or structure, or as an ecosystem. Yet, the remaining areas of 'nature' (i.e. streams, parks and other green spaces) are crucial for human health and are interlinked with the urban ecosystem. Case studies have shown that exposure to and understanding of ecosystems and nature can improve city life (Rees 2003). For example, Ulrich (1984) showed that time for recovery from an injury significantly decreased when individuals were exposed to nature simply from the viewing of nature through their hospital bed window. The increasing number of people living in urban areas means that many children first encounter nature playing in urban streams. Improving the ecological value of urban streams, and hence the opportunity to have a connection to interact with a healthy ecosystem within a metropolitan area, provides recreational, cultural and aesthetic enjoyment for urban dwellers (Meyer *et al.* 2005).

In this thesis, the issue of the impact of metals on the ecological health of our urban streams will be investigated and discussed. The major contributing factors to the degradation of urban streams and the effects on the organisms that reside in our urban streams will be introduced. In particular, the effects of heavy metals to their receiving aquatic environment and how these pollutants are managed. It is important to manage the effects of stormwater into these urban streams not only for ecological reasons, but to offer city dwellers a glimpse of nature and provide opportunities for recreational, cultural and aesthetic enjoyment, all contributing to the quality of urban life (Meyer *et al.* 2005).

1.1 Urban stream syndrome

Globally, urban streams often have poor water quality, are physically and hydrologically degraded, and have been described as suffering from 'urban stream syndrome' (Walsh *et al.* 2005). This syndrome is largely caused by increases in impervious surfaces which transport contaminated stormwater into streams. Symptoms of the syndrome include a flashier hydrograph, elevated concentrations of nutrients and contaminants, altered channel morphology, and reduced biotic richness, with increased dominance of tolerant species (Walsh *et al.* 2005). As little as 6 - 10% impervious cover has been shown to result in a detrimental impact on streams (Klein 1979; Walsh *et al.* 2005; Paul and Meyer 2008). The increase in impervious surfaces leads to decreased infiltration and increased surface runoff (Walsh *et al.* 2005). The impervious cover model classifies streams into one of three categories: sensitive, impacted and non-supporting/urban drainage (Figure 1.1) (Schueler *et al.* 2009). While there are limitations to this model, it is based on numerous studies which ultimately show that there is a level of degradation at very low percentages of impervious cover (Walsh *et al.* 2005). As a result, impervious cover has become a useful predictor of urbanisation and urban impacts on streams (Klein 1979; McMahon and Cuffney 2000).

In conjunction with increased impervious cover, many urban streams also have altered channel morphology and stability as a result of drainage systems. This also results in flashy hydrographs, elevated concentrations of nutrients and contaminants, and therefore reduced species richness and community composition (Meyer *et al.* 2005; Walsh *et al.* 2005; Mills 2008). While these symptoms show consistent increases or decreases with urban land use, they may differ in the degree to which they change depending on the level of urbanisation and other aspects of the surrounding environment, such as riparian vegetation (Walsh *et al.* 2005). The Urban stream syndrome is a product of multiple effects which can be cumulative and synergistic. Understanding these impacts and unravelling the contribution that individual stressors make to the overall impact is difficult, making the approach to remediation complex (Kelly 2010).

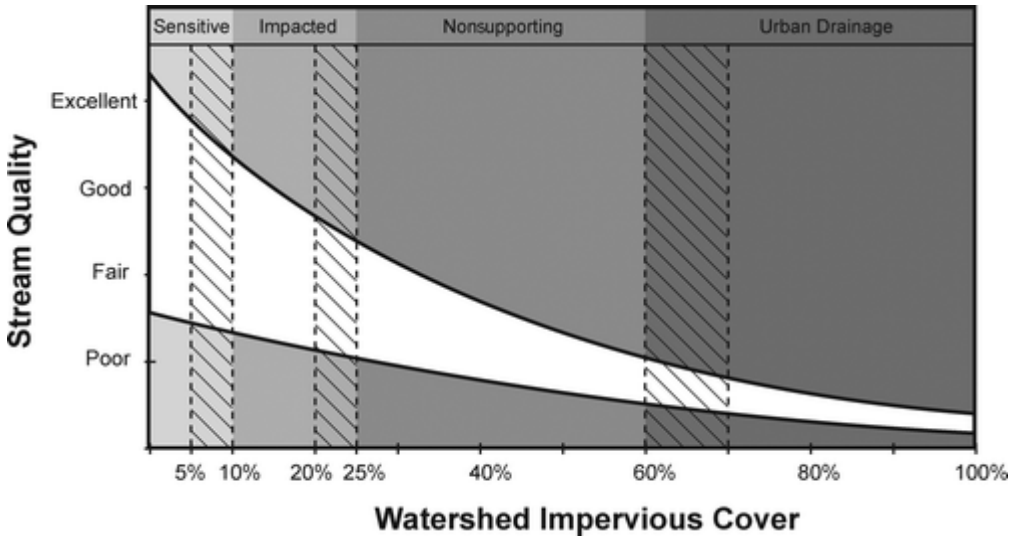

Figure 1.1: The relationship between impervious cover and water quality as defined by Schueler et al. (2009)

Chemical characteristics are much more variable than those of hydrology or geomorphic nature. They are dependent on the type of urbanisation, whether it is industrial or residential, what type of stormwater drainage there is, and whether this includes wastewater treatment plants (WWTP) or combined sewer overflows (CSO) (Paul and Meyer 2008). In general, there is an increase in almost all physico-chemical parameters such as oxygen demand, conductivity, suspended solids, hydrocarbons and metals in urban streams (Porcella and Sorensen 1980; Lenat and Crawford 1994) . Globally, changes in WWTP processes and technologies have resulted in significant reductions in chemical contaminants. In New Zealand, many point-source problems such as WWTP have been significantly improved, however, as there are still CSOs present in urban streams across the country these can still cause issues during storm events. Hnatukova *et al*. (2009) showed that the CSOs cause significant deterioration of sediment quality of the streams. In particular, that levels of metals increase with sewer overflow outputs. Yet, non-point source (NPS) discharges possibly still prove to be a more challenging and perhaps more worrisome issue. The cumulative effects and ubiquitous nature of NPS problems is recognised as a key factor responsible for the overall biological degradation in urban streams (Gnecco *et al.* 2005; Fraga *et al.* 2016).

The designation of stormwater as point or non-point source is problematic and it is not clearly defined under the Resource Management Act (1991). Stormwater can be considered as point source, as it is often channelled into storm drain systems and therefore, discharged through a point source (pipes) into streams. However, unlike other types of point source, such as WWTPs and CSOs, stormwater cannot be attributed to a single activity or one specific area. Therefore, in terms of regulating activity and effective

treatment, stormwater can be considered as non-point pollution resulting from urban surface runoff as reflected in much of the literature (Lee and Bang 2000; Gnecco *et al.* 2005).

Rain water washes off the dissolved, colloidal and solid constituents from urban surfaces which include a number of contaminants into receiving waterways (Gnecco *et al.* 2005; Walsh *et al.* 2005; Zhang *et al.* 2010). This pollutant load is largely sourced from vehicular traffic and materials used in built environments such as galvanised roofs (Davis *et al.* 2001; Zhang *et al.* 2010; Davis 2010). This pollution load is not necessarily predictable or consistent. Stormwater from different catchments has been shown to contain different concentrations of pollutants dependant on the differing functional areas of roofs and roads discharging into the aquatic system (Zhang *et al.* 2010). In addition, the loading of pollutants is also not consistent over time, even within the same rainfall event. This is because runoff during the 'first flush' of a storm bears the highest concentration of pollutants (Bertrand-Krajewski *et al.* 1998; Lee *et al.* 2002). As much as 80% of pollutant load has been shown to be carried by the 'first flush' event, which is the first 30% of runoff volume. (Bertrand-Krajewski *et al.* 1998). Therefore, management of stormwater is problematic, firstly with the differing pollutants and also the differing loads. Management practices must be capable of dealing with episodic events with high loads.

1.2 Invertebrates in urban streams

Aquatic benthic invertebrates in streams are often used as biological indicators of stream health. They are relatively long-lived, not washed away by small floods and not as migratory as fish, making it possible to characterise their communities in streams with a reasonable level of accuracy (Miserendino *et al.* 2008; Shaver and Suren 2011). Although there are invertebrate taxa that are tolerant of pollutants, there are also sensitive taxa, for example the orders Ephemeroptera, Plecoptera and Trichoptera (EPT taxa). These organisms are relatively easy to identify and provide a measure that translates to the health of the aquatic system (e.g. %EPT) (NZ Transport Agency 2009). The difference in sensitivity to pollutants across the benthic macroinvertebrate community hence provides a graded response to a broad range of types and degrees of stress (Beasley and Kneale 2002).

Substantial literature exists on the response of benthic invertebrates to impacts in urban streams. Specifically, effects of organic pollutants on invertebrates has been historically well studied (Paul and Meyer 2008). Invertebrate response to toxins, siltation, temperature change and organic and inorganic nutrients have also been well studied. Overall, the responses by the invertebrates can be summarised as decreased diversity and abundance. As expected, decreases are especially evident in the sensitive

orders—EPT taxa (Pratt *et al.* 1981) . Paul & Meyer (2001) summarised studies in the USA and found that invertebrate diversity sharply decreased between 1% and 33% impervious cover. Most studies have also observed decreases in overall invertebrate abundance with increasing impervious area, however, the relative abundance of Chironomidae (non-biting midges) and Oligochaetes tend to increase (Pratt *et al.* 1981; Thorne *et al.* 2000).

1.3 Heavy metal source, fate and toxicity

Heavy metals are defined as metallic elements that have relatively high density compared to water (Tchounwou *et al.* 2012). Given that metalloids (elements with properties intermediate between metals and non-metals), such as arsenic (As), are able to induce toxicity at low level exposure; the term heavy metals often includes metalloids and will be used throughout this thesis. Some metals are essential micronutrients such as manganese (Mn), zinc (Zn) and iron (Fe). They are essential to life in the right concentrations, but in excess, they can become toxic (Beasley and Kneale 2002; Harding 2005). Chronic low exposures to some metals can also cause toxicity in organisms. One of the most crucial properties of metals that separates them from other toxicants is that they are not biodegradable in the environment (Beasley and Kneale 2002; Davis 2010). This means that accumulation of heavy metals in the environment can cause significant negative impacts.

1.3.1 Sources of metals

Heavy metals naturally occur throughout the earth's crust and trace amounts will always be present in fresh waters (Sekabira *et al.* 2010). Environmental contamination is viewed as an 'exceedance' of the natural concentrations is predominantly a result of anthropogenic activities. Contaminant point sources such as mining, smelters and other metal-based industrial operations can cause significant ecological damage due to pollutants being directly discharged. However, non-point source contamination is arguably a more significant issue as solutions are more complex (Lee and Bang 2000). In the urban area, stormwater is a major source of pollution and within that, the major source of heavy metals in urban streams.

Road and roof runoff are the major sources of metals in stormwater (Davis *et al.* 2001) Metals accumulated on roads, parking lots, and other impervious surfaces are transported to neighbouring waterways through stormwater by rainfall. While there are emerging contaminants that have recently come into focus, metals continue to dominate stormwater signatures (Wicke *et al.* 2012). Due to their persistence in the environment, metal concentrations have and will likely continue to increase in aquatic ecosystems as a result of human production and consumption activities (Beasley and Kneale 2002).

While there are many metals that are potential contaminants and toxic to aquatic life, the majority of research has focussed on cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn). This is predominantly because these metals are the most ubiquitous in stormwater runoff. Lead is largely a historic issue, where there is a legacy effect from the use in petrol and paint. However, it is still used in some paints, emitted from brake wear and tyre dust and is also present in roof runoff (Beasley and Kneale 2002; Mills 2008; Yu *et al.* 2014). In contrast, galvanised roofs are a major source of Zn and are especially a problem in older urban areas where the roofs are not painted or the paint is degrading (Mills 2008). Roof runoff typically contains elevated concentrations of trace metals such as Cd, Cu, Pb, and especially Zn. These metals leach from weathered metal roofing material and are present in deposited particles on the roof surface (Pennington and Webster-Brown 2008). The fine material released from brake and tyre wear is also a primary source of Zn (Beasley and Kneale 2002). Tyre and brake pads from New Zealand vehicles have concentrations of Zn in the range 1190 – 18,300 mg/kg (Zander 2005). Vehicle brake pads and guttering are the major sources of Cu (Pennington and Webster-Brown 2008). Dust from brake pads contain 346 – 9630 mg Zn/kg and 70 – 1980 mg Cu/kg (Zanders 2005), causing Zn and Cu to be the most prevalent metals in stormwater (Mills 2008). These heavy metal contaminants have been the principal concern of research to date. It is important to consider that there are a range of other metals that are used in industry and are likely to be present in urban environments. These include antimony (Sb), nickel (Ni) and silver (Ag) from brake linings (Kennedy 1999; Hjortenkrans 2007), and nickel (Ni) and cobalt (Co) used in industrial activities such as electroplating and galvanising.

1.3.2 Fate and toxicity of metals

Heavy metals can be found in streams in many different forms, depending on their source and interaction with other complexes. Their toxicity and bioavailability is largely determined by the state in which the metal is present. Metals in their free (dissolved) state are easily taken up by organisms through respiration across gill surfaces and often have high toxicity in this form (EPA Victoria 2013). Metals can also be present as complex molecules with carbonate, chloride, or dissolved organic matter, where toxicity is greatly reduced even when still dissolved (EPA Victoria 2013). Suspended solids, which carry a significant portion of trace elements transported by stormwater, settle on the streambed and accumulate in sediments (Hnatukova *et al.* 2009; Sekabira *et al.* 2010). The concentration, storage, and transport of trace elements are also dependant on particulate organic matter content and sediment characteristics (Hnatukova *et al.* 2009; EPA Victoria 2013). For example, organic matter has a high binding affinity to metals and thus, concentrations can increase greatly in sediments which are high in organic matter content (Paul and Meyer 2008; EPA Victoria 2013). Although most pollutants adsorbed on sediments are not readily

available to aquatic organisms, variation in chemical properties in the stream such as pH, may induce the release of metals from the sediment to the pore water or overlying water (Hnatukova *et al.* 2009).

While high metal concentrations can be directly toxic to invertebrates and fish, metals can also accumulate in plants and sediment and be consumed and enter the stream food chain. Therefore, species feeding characteristics, trophic interactions, and biochemical/physiological adaptation also play an important role in the bioavailability of trace metals (Tchounwou *et al.* 2012). Greig *et al.* (2010) showed that streams with naturally high metals had very low fish biomass, while naturally acidic streams showed little effect, demonstrating that metals are highly toxic to fish species.

The mechanism of the effects of metals on benthic invertebrates are not as clear compared to fish. While strong responses on invertebrate communities are observed in metal contaminated streams, these results have not been supported by 'traditional' laboratory toxicity tests. Concentrations of order of magnitudes higher than found in the environment are required to achieve mortality in laboratory trials (Poteat and Buchwalter 2014). At least two factors may be responsible for this pattern, 1) reaching steady state tissue concentrations takes longer than the duration of ecotoxicity trials, and 2) metals obtained from diet are more important in tissue burden and physiological activity rather than dissolved metals (Poteat and Buchwalter 2014). The traditional understanding of dissolved acute toxicity mechanisms is derived from studies of fish. Where surface action of metals on gills cause osmoregulatory disturbances, causing them to be highly sensitive to metals (Poteat and Buchwalter 2014). Benthic invertebrates do not appear to display the same mechanisms. Bioaccumulation of metals by benthic invertebrates has been shown to be more important from the diet rather than being taken up directly from the water via the dissolved route of exposure. In addition, some studies have shown that dietary exposure resulted in significant depression of antioxidant enzymes, which protect against free-radical induced cell damage, whereas dissolved exposure did not (Xie and Buchwalter 2011). Performing traditional acute toxicity tests, therefore, may not consider the most probable main pathway of exposure or the amount of time required for effects to be observed.

Research to date has included investigations of the relationship between metal concentrations in sediment and bioaccumulation in benthic invertebrates (Beasley and Kneale 2002; de Paiva Magalhães *et al.* 2015) and comparing bioaccumulation between feeding groups (Cid *et al.* 2010; Kolaříková *et al.* 2012). From these studies, it has been consistently shown that bioaccumulation varies widely between species living and feeding in the same habitat (Luoma and Rainbow 2005; Cid *et al.* 2010). Bioaccumulation is also

dependent on the specific metal (Cid *et al.* 2010), hence more studies on the toxicity of differing metals to benthic invertebrates are needed.

1.4 New Zealand urban streams

Internationally, New Zealand is marketing itself as '100% pure', giving an image of a clean, green and pristine country, however the reality is that many of our lowland rivers are in poor ecological condition (e.g., 96% of lowland rivers are not swimmable) (Joy 2014). The quality of New Zealand rivers and freshwater systems has become an important issue for the public and gaining considerable media attention as the situation worsens (Chapman 2015; Mitchell 2015). Although urban areas in New Zealand make up less than 1% of New Zealand's total land cover, urban streams are arguably the most degraded and very visible to the public (van Bunnik 2007). Furthermore, with most major urban areas either being coastal or associated with rivers, many major streams and outlets into estuaries and coastal waters are significantly impacted.

New Zealand has 16 main urban areas, defined as areas with greater than 150 people per km^2 (Statistics New Zealand. 2001). The focus of this thesis is on the three main urban centres; Auckland, Christchurch, and Wellington. Few studies of heavy metal concentrations in sediment and/or water have been investigated in urban streams for the three cities (O'Sullivan *et al.* 2012; Alsager 2012; Ancion *et al.* 2013). In addition, there has not been a study comparing multiple cities and whether trends are consistent across urban areas in New Zealand. Council data from Auckland, Wellington, and Christchurch have all reported Cu, Pb, and Zn exceeding ANZECC water quality guidelines within their respective urban catchments (Heath *et al.* 2014; Lockie 2014; Margetts and Marshall 2015). Where sediment has been monitored, this too has exceeded guidelines for Cu, Pb, and Zn (Golder Associates 2012). It is important to understand heavy metal concentrations and trends in urban areas for effective management purposes. The results of the limited available data highlight the relevance and importance of understanding the effects of heavy metals on the benthic invertebrates in New Zealand urban streams.

Benthic invertebrate data is often collected by the associated city councils, however, again there has not been any large scale comparisons of urban streams encompassing different urban areas. Most studies have focussed on a single catchment or stream, making it difficult to determine relationships between stressors and responses on the large scale. Ultimately, without intervention, these urban streams may result in being modified to the point where they provide very little, if any, ecological function (Kelly 2010).

This has direct consequences for social, economic, and cultural effects on society. These range from the aesthetic value of the water body being diminished due to pollution (potentially resulting in decreased property values), to the cultural effects on Māori, the water body losing its mauri (life-force) and mahinga kai (food gathering) values (Pauling 2007).

1.5 Stormwater management

Historically stormwater and wastewater management in New Zealand, has involved altering streams into drains or sewers. Thus with the growing understanding of the affect that stormwater has on the receiving aquatic environment, there is a paradigm shift occurring to integrated and inclusive catchment management. There is also a challenge to shift communities away from associating value with for example, mowed grass riparian zones or paved streamside paths and to educate how streams more closely resembling natural conditions might be more desirable (Walsh *et al.* 2005).

Managing and restoring urban streams requires a thorough understanding of the biological values that can realistically be achieved given the challenges and the streams physical and biological characteristics (Mills 2008). All aspects of restoration must also be considered, for example, while riparian management could substantially improve stream health, full protection would not necessarily be achieved without also managing the water quality, hence the need for integrated catchment management (Mills 2008). To achieve this, well researched and understood data and information is required. In terms of metal contamination, which can be a large constituent of stormwater, it could be argued that there is not yet sufficient understanding of the effects on the benthic invertebrate community to determine the most effective management plan both globally and specifically in New Zealand.

The Resource Management Act (RMA) (1991) is New Zealand's primary legislation of environmental management. It influences policy around the use, development and protection of its natural and physical resources through effects-based legislation. Section 5 of the RMA requires:

> "safe-guarding the life-supporting capacity of air, water, soil and ecosystems"

Thus, the Act works to prevent any person from discharging a contaminant into water, or onto land that is likely to enter water, unless allowed by a regional plan or resource consent.

New Zealand has recently released the National Policy Statement on Freshwater Management (NPS-FM) (2014) which includes objectives on contaminants and flow of streams (Ministry for the Environment 2014). City and district Councils also have to apply for discharge consents from regional councils for stormwater, requiring an Assessment of Environmental Effects (AEEs), generally resulting in conditions of

consent including monitoring and the development of stormwater management plans. While there is no direct requirement by the NPS-FM to ensure metal concentrations do not reach toxic concentrations, the values and objectives made by regional councils should encompass this. Each city therefore has their own approach to managing the level of contamination.

1.6 Māori cultural values

Māori regard freshwater as highly significant. The ever increasing degradation of rivers results in the loss of their mauri (life-force), meaning they are unsuitable for cultural practices, specifically mahinga kai. The translation of mahinga kai literally means 'food works' (Tipa and Nelson 2008). The term encompasses 'the ability to access the resource, the site where gathering occurs, the act of gathering and using the resource, and the presence and good health of resources' (Tipa and Nelson 2008). However, for the purpose of this research, mahinga kai will be referred to in terms of mahinga kai in freshwater systems and more specifically the areas that indigenous fish and other freshwater species are, or were, gathered as food sources and the indigenous mahinga kai species themselves. These species include banded kokopu, inanga, koura, and tuna (NIWA 2013).

There is an inherent connection between Māori and the natural environment (in particular waterways) that makes the management of these environments of paramount interest and priority (Harmsworth 2013). These resources are often crucial for Māori to continue to transfer the knowledge of their tīpuna (ancestors) to future generations and retain their cultural identity. The values of freshwater are described by Māori as a resource for Māori communities through the capacity of healthy waterbodies to provide food, resources and opportunities to maintain traditional connections and practices (Panelli and Tipa 2009).

Te Rūnanga o Ngāi Tahu (TRoNT) have undertaken cultural assessments of the catchments in Christchurch (The State of the Takiwa reports). These assessments integrate Mātauranga Māori (Māori tradittional knowledge) with western science providing an environmental monitoring and reporting process that takes into account tāngata whenua values (TRoNT 2001). Results from assessments undertaken in 2007 found none of the surveyed waterways could be classified as being 'good' or 'very good' condition with only three out of 30 sites considered good enough to return to for mahinga kai practices (Pauling 2007). While similar cultural environmental health assessments have been conducted by Māori in other areas of New Zealand, it appears that none have been undertaken in either Wellington or Auckland regions.

As mentioned above, benthic invertebrates are widely considered to be valuable indicators of stream health due to their ubiquitous and relatively long life cycles. They are also the main food source for fish, therefore understanding how invertebrates are affected will improve our understanding of stream food chains and the implications for mahinga kai. Conducting research on invertebrates could inform stream management approaches and restoring mahinga kai practices and species.

1.7 Thesis Objectives

The objectives of this thesis were to:

- Investigate heavy metal contamination and overall benthic inverterbate health in streams in three of New Zealand's major urban centres; Auckland, Christchurch and Wellington.
- Investigate relationships between metal contamination and invertebrate community structure.
- Determine any differences between cities in overall metal contamination and benthic invertebrate communities of urban streams.
- Compare bioaccumulation of Zn and Cu exposure through biofilm and dissolved exposure by the *Deleatidium* spp. mayfly.
- Compare bioaccumulation of Zn and Cu as sinlge metal species and as a mixture through biofilm and dissolved uptake by the *Deleatidium* spp. mayfly.

1.8 Thesis Structure

Chapter one provides background to the research and general state of urban streams globally as well as in New Zealand. Current literature on the effects of metal contamination on benthic macroinvertebrate communites is reviewed. Chapter two and three describe a one-off survey undertaken in 10 urban streams each in Auckland, Wellington, and Christchurch investigating the level of heavy metal contamination and associated macroinvertebrate communities in these urban centres. Chapter four describes laboratory studies performed to determine the differences in Cu and Zn uptake as single metal species and as a mixture by *Deleatidium* spp. through diet and water exposure. Chapter five is a discussion of the key findings and potential implications for management of urban waterways and mahinga kai activities.

2 Chapter 2: Heavy metals in stream water and sediments in three cities

2.1 Introduction

Urban runoff is a major source of heavy metals in urban streams. Heavy metals are frequently investigated due to their toxicity, ubiquitousness and persistence (Beasley and Kneale 2002; Davis 2010). The majority of studies on heavy metals in stormwater and urban streams have focussed on copper (Cu), lead (Pb), and zinc (Zn). This study will investigate a wider suite of heavy metals to provide a thorough understanding of the level of contamination in urban streams.

The three major urban cities in New Zealand; Auckland, Christchurch, and Wellington are the focus of this study. Each of these cities lies on the coast and have many streams making their way through the city before flowing into the ocean. A total population of 1,453,800 live in Auckland (Statistics New Zealand). The Auckland region has 21,000 km of rivers and streams of which 8% are within the urban area. In contrast, Christchurch's population is 381,800 (Statistics New Zealand) with a total of 355 km of streams as well as 500 km of stormwater pipes within the city boundaries. Wellington has a similar population to Christchurch with 398,200 in the urban area (Statistics New Zealand). There are 12,300 km of rivers in the greater Wellington region, while the proportion of this in the Wellington urban area is not reported, the urban land area is much smaller than that of Christchurch and Auckland. Stormwater pollution is a concern for all of the these cities and regional councils, with each having a monitoring program and stormwater management plans currently in place and new plans in the process of being developed.

There are limited studies of heavy metals in urban streams outside of council monitoring. Auckland Council monitors total and soluble Pb, Cu and Zn at a range of sites. The median concentrations generally exceed ANZECC water quality guidelines for Cu and Zn, but rarely for Pb. Heavy metals in sediments are not routinely monitored and only Cu, Pb and Zn are monitored in the water column (Lockie 2014).

Christchurch City Council performs monthly monitoring of Cu, Pb, and Zn in the water at over 40 sites. Heavy metals in the sediments are also monitored at different catchments on a five yearly cycle. The latest sampling round (January – December 2014) for Christchurch City Council showed limited exceedance of dissolved Cu across all catchments, with only one site to record a median above the recommended guideline. While median levels of all sites for dissolved Zn were not exceeded, it was not uncommon for individual sampling events to exceed guidelines (Margetts and Marshall 2015). Sediment Zn most commonly exceeded ISQG-low value followed by Pb then Cu (Golder Associates 2012).

The Greater Wellington Regional Council conducts annual sampling of 10 urban streams sites for dissolved Cu, Pb, and Zn. Water samples are analysed for a wider range of metals approximately every five years and sediment concentrations are occasionally reported (Milne 2008; Morar 2013). Three out of the 10 sites they monitored exceed ANZECC guidelines for dissolved Cu at least 50% of the time and three out of the 10 also exceeded ANZECC guidelines for dissolved Zn (Heath *et al.* 2014).

Literature outside of council reports include Blakely and Harding (2005); O'Sullivan *et al.* (2012) in the Christchurch urban region, both of which showed sediment and water samples significantly exceeded ANZECC guidelines. These studies were performed at different sites than the council monitoring sites. Davis *et al* (2010) reported on long-term monitoring in the Auckland region and identified Cu, Pb and Zn as metals of concern.

The monitoring by city councils is not consistent between cities, and data for sediment or heavy metals that are not Cu, Pb, or Zn are limited. Furthermore, literature in New Zealand on heavy metals in urban streams is largely reported for single streams or a single catchment. This study sampled water and sediment from a range of sites within Auckland, Christchurch, and Wellington for analysis of heavy metals. Therefore, this study is the first where metal concentrations in the water and sediment can be compared across the three major urban centres in New Zealand.

The aims of the heavy metals investigation were to:

- Determine the level of heavy metal contamination in urban streams in Auckland, Christchurch, and Wellington and compare between the cities.
- Determine if there is a relationship between physical characteristics of the catchments and concentrations of heavy metals.

2.2 Methodology

2.2.1 Consultation with tangata whenua

As part of the research approach, consultation with iwi was undertaken prior to conducting fieldwork. The aim of the consultation was to inform the hāpū, rūnanga or iwi of the study in their takiwā (region) as an acknowledgement of their status as manawhenua and to provide an opportunity to voice any concerns or suggestions on the research. Importantly, the purpose was also to get support and endorsement of the research. The consultation process with local iwi and rūnanga differed between cities reflecting the number of entities required to contact and the ability to contact them. In Christchurch a meeting kanohi ki te kanohi (face to face) was organised and held at Tuahiwi Marae in March 2015 with the environmental and cultural advisors for Te Ngāi Tuahiriri Rūnanga, who are the kaitiaki (guardians) of the urban area. Approval and endorsement was given and areas of interest to the rūnanga were identified (see Appendix 4 for letter). The Wellington iwi groups were identified from the Greater Wellington Regional Council website and phoned to inform them of the proposed research, correspondence was continued with those interested. Due to the large number of iwi in the Auckland region (19) and the larger urban area, sample sites were determined and iwi that had interest in the relevant areas (14 iwi) were e-mailed, again, correspondence was pursued with those that were interested.

2.2.2 Study sites

A one-off survey was conducted in three of the major urban centres of New Zealand; Auckland, Wellington, and Christchurch. Local knowledge and the use of existing city council biomonitoring sites were used to identify potential streams that would represent a gradient of low to high concentrations of heavy metals. Targeted areas included areas with low impervious cover, impacted by industrial activities, and sites of interest to tangata whenua in the area. At many sites, previous data on metal concentrations were absent or limited, therefore land-use upstream of the potential sampling sites was used as selection criteria. A number of potential sites were identified in each urban centre. On visiting these sites, acceptability of the sites was determined based on physical accessability, stream size (i.e. wetted width <4m) and wadeability. Ten streams from each urban centre were selected to be sampled on a single occasion (GPS co-ordinates of sites in Appendix 1 Table 6.1) At each site a sampling reach of approximately 20 m was selected. Where possible the reach included a riffle-run-pool complex, but often pools were not present. In general, sampled reaches were upstream of road crossings and not immediately downstream from tributaries.

2.2.3 Physico-chemical characteristics

In the field, basic water chemistry parameters including pH, dissolved oxygen (% and mg L^{-1}), temperature, and specific conductivity were measured at each site using hand held meters (YSI 550 and 63). Depth was also measured at five equidistant intervals across the wetted width of the stream. Surface velocity was measured by timing a float to travel two metres and calculating from v = d/t; this was repeated three times in order to obtain an average.

Physical habitat conditions were assessed at each site using the P1 site characterisation field sheet (Appendix 5) (Harding 2009). Substrate composition was determined by visual assessment of 30 substrate samples at regular intervals across the stream using the Wentworth scale. The Substrate Index (SI) was calculated from substrate composition using the equation adopted from (Jowett *et al.* 1991):

$$SI = 0.08\%bedrock + 0.07\%boulder + 0.06\%cobble + 0.05\%gravel + 0.04\%fine\ gravel + 0.03\%sand \quad \textit{Equation (2.1)}$$

where derived values for the substrate index range from 0 (i.e. a substrate of 100% silt) to 8 (i.e. a substrate of 100% bedrock); the larger the index, the coarser the overall substrate. In general, coarser substrate (up to cobbles) represented better instream habitat than finer substrate.

The physical and chemical characteristics of the sample sites in each region are presented in Table 2.1.

Table 2.1: Range of physico-chemical parameter and field spot measurements for 10 streams in each city. Mean in parentheses. Individual site raw data is in Appendix 1 Table 6.4. SI = substrate index

	SI	Depth (m)	Width (m)	Velocity (m s^{-1})	pH	Specific Conductivity (μS_{25} cm^{-1})	Temperature (°C)	DO (mg L^{-1})
Auckland	0.0 - 5.6 (3.2)	0.02 - 0.40 (0.16)	0.80 - 3.20 (2.02)	0.02 - 0.73 (0.23)	6.1 - 9.4	174 - 905 (310)	8.9 - 16.3 (12.6)	6.6 - 7.6 (7.5)
Christchurch	2.0 - 5.1 (4.3)	0.04 - 0.32 (0.16)	1.00 - 5.00 (2.53)	0 - 0.48 (0.23)	6.5 - 7.4	106 - 270 (176)	9.4 - 15.9 (13.6)	5.1 - 10.0 (7.5)
Wellington	4.7 - 5.6 (5.2)	0.04 - 0.21 (0.10)	1.00 - 6.20 (2.90)	0.10 - 0.69 (0.36)	6.9 - 8.2	193 - 325 (241)	7.1 - 10.1 (8.8)	4.9 - 12.0 (10.8)

2.2.4 Sample collection

Water chemistry

In Christchurch, spot water samples were collected in 50 mL Falcon tubes at each site and stored on ice until returned to the laboratory. Total and dissolved metals were analysed from the same water sample, with 10 mL of sample water filtered through a 0.45µm membrane filter (Millex HA (33mm) sterile filter unit) to determine the concentration of dissolved metals. Ultra-pure concentrated HNO_3 was added (50 µL of acid per 10 mL of sample) to both total and dissolved samples for preservation until analysis.

In Wellington and Auckland, water samples were either collected directly into 50 mL Falcon tubes for total metal concentration or filtered on site (through 0. 45µm filters) into 50 mL Falcon tubes for dissolved metal concentrations. Both samples were then stored at 4°C until return to Christchurch. Acid (HNO_3) was added to the samples and the samples left to stand for one week before analysis.

Milli-Q 50 mL blank water samples were taken for each day of field work and exposed to the environment for the approximate time of collecting a sample. This was to control for any transport and environmental effects. All blanks were below detection limits (Table 2.2).

Sediment Samples

A composite sediment sample was collected at each site by scooping from the top 2 cm of the stream bed from multiple locations within the reach into new 200 mL plastic containers. The samples were stored on ice in the field and then frozen until drying and digestion for metal analysis in the laboratory. Sediment samples could not be collected at all sites due to streams either being concreted or very little sediment.

In the laboratory, sediment samples were dried at 70°C to remove moisture and kill any harmful pathogens that may be present. Each sample was disaggregated in double bagged zip lock bags with a metal rolling pin and sieved into two size fractions - < 2mm and < 63µm. One gram of each sieved sample was weighed into new 50 mL polycarbonate vials for digestion. The samples were digested using a modification of US EPA 200.8 (US EPA 1994), 4 ml of nitric acid (1+1) and 10 ml of hydrochloric acid (1+4) were added to the 1 g sample in the digestion tubes and left to stand overnight. The samples were heated in heating blocks at 85°C for 10 – 15 minute until refluxing and heating continued for 45 minutes. After cooling overnight (for at least 12 hours), they were made up to 20 ml with Milli-Q water and again left overnight to settle out particulates. Each sample was then diluted 21x with 2% HNO_3 for ICP-MS analysis. A certified marine reference sediment material (CRM) (U.S. National Institute of Standards and Technology Standard Reference Material 2702) and blanks were included with each digestion batch. Recoveries for the CRM standard reference material are presented in Table 2.3.

The sediment digestion method was modified due to the amount of < 63 µm sediment available for analysis. For most samples 0.5 g was available and the acid volumes were adjusted accordingly.

2.2.5 ICP-MS procedure

Water samples were analysed by Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) using an Agilent 7500 Series ICP-MS with an octopole reaction system at the University of Canterbury. A water certified reference material, IV SRm 1643, was analysed with each analytical run and an internal standard of Rh was added online. The isotopes analysed were ^{107}Ag, ^{27}Al, ^{75}As, ^{44}Ca, ^{111}Cd, ^{59}Co, ^{53}Cr, ^{63}Cu, ^{57}Fe, ^{24}Mg, ^{55}Mn, ^{60}Ni, ^{208}Pb, ^{121}Sb, ^{51}V, ^{66}Zn.

2.2.6 Quality Assurance/Quality Control

The QA/QC data for the ICP-MS water analysis and the sediment digestions are presented in Tables 2.2 and 2.3. Filtered and unfiltered milli-Q blanks were analysed with the water samples. The sediment digestions included blanks and were also analysed by ICP-MS. Certified standards and duplicates were analysed in both the water and sediment analyses.

Table 2.2: Water samples detection limits, recoveries for IV SRM 1643, and blanks.

	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Ag	Cd	Sb	Pb
Detection limits (µg L^{-1})	1.0	0.1	0.1	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1	0.1	0.1
IV SRM 1643 % Recovery (n=3)	83.9	88.2	86.0	90.7	67.6	90.6	93.4	90.2	97.9	91.0	89.5	98.0	85.1	96.3
Blanks	1.2	<0.1	<0.1	<0.1	<1.0	<0.1	<1.0	<1.0	<1.0	<0.1	<1.0	<0.1	<0.1	<0.1

Table 2.3: Sediment samples detection limits, CRM recoveries, and blanks.

	V	Cr	Fe	Co	Ni	Cu	Zn	As	Ag	Cd	Sb	Pb
Detection limits (µg g^{-1})	0.1	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1	0.1	0.1
<2 mm												
IV SRM 1643 % Recovery (n=1)	82.2	77.4	65.5	85.6	90.0	87.7	93.9	87.1	95.5	96.2	80.1	83.6
Blanks	0.14	0.36	20.98	<0.1	<1.0	<0.1	<1.0	<0.1	<0.1	<0.1	<0.1	<0.1
Sediment CRM[1] % Recovery (n=2)	65.5	60.3	-	73.2	56.1	73.5	76.2	81.5	101.3	96.7	15.0	100.9
<63 µm												
IV SRM 1643 % Recovery (n=1)	96.5	96.6	87.3	94.0	99.7	86.6	94.5	92.3	-	94.1	78.2	98.1
Blanks	0.3	<0.1	9.9	<0.1	<0.1	<0.1	1.4	<0.1	-	<0.1	<0.1	<0.1

[1]U.S. NIST certified marine sediment Reference Material 2702

2.2.7 Organic matter

Loss on ignition was used to provide a measure of organic matter in the sediments at each site. The method from Dean Jr (1974) was modified. Approximately 10 g of the dried sediments was weighed and placed in pre-weighed and cleaned silica dishes. Samples were heated in muffle-furnace at 550°C for 4 h and left in the muffle-furnace overnight to cool. Once cooled, samples were weighed again and the loss in mass converted to a percent organic matter (%OM).

2.2.8 Catchment conditions - Impervious surface area

Impervious surface area is often used as a metric of urban intensity (Morse *et al.* 2003). The proportion of impervious area for each sites corresponding catchment was extracted from the FENZ (Freshwater Ecosystems of New Zealand) dataset using ArcGIS 10.4.

2.2.9 Data analysis

Water Pollution indices

The **cumulative criterion unit** (CCU) is a metric developed by Clements *et al.* (2000) as a cumulative measure of metal concentrations to examine the relationship between benthic community structure and heavy metals. Interactions among metals are assumed to be additive and the CCU is defined as the ratio of the measured metal concentration to the U.S. EPA criterion value, summed for all metals at a site. The water hardness was calculated from Ca and Mg concentrations to determine the criterion values (c_i) and therefore, the CCU.

$$CCU = \sum m_i/c_i \qquad \textit{Equation (2.2)}$$

where m_i is the measured metal concentration and c_i is the hardness based criterion value for the *i*th metal. C_i was determined from the US EPA Continuous Criterion Concentrations (CCC) and water hardness (US EPA. 2016). The CCU has four categories for level of contamination, values <1 are considered to be background concentrations, 1 – 2 low metal contamination, 2 – 10 medium metal contamination, and >10 high metal contamination.

Sediment pollution indices

Two sediment pollution indices were calculated in order to compare metal contamination in the stream sediment of the three cities. Background heavy metal concentrations at each site were required to calculate these indices. Background heavy metal concentrations were available for Auckland, Christchurch and Wellington from councils for the different soil types found in each region (ARC 2001; Sulzberger and Whitty 2003; Tonkin & Taylor 2007). Where concentration ranges were provided, the maximum background value is used. Background heavy metal concentrations for each of the soil types sampled are presented in Table 2.4.

Table 2.4: Background heavy metal concentrations in soils for Auckland, Christchurch, and Wellington. Only relevant soils are shown. All units are mg kg^{-1}.

		As	Cd	Cr	Cu	Ni	Pb	Zn
Auckland	Volcanic	12.0	0.7	125.0	90.0	320.0	65.0	1160
	Non-Volcanic	12.0	0.7	55.0	45.0	35.0	65.0	180
Christchurch	Gley	10.6	0.2	18.5	23.3	15.6	34.9	138
	Recent	15.3	0.2	19.0	17.7	16.6	101	149
Wellington	Greywacke	7.0	0.1	16.0	25.0	13.0	78.6	105
	Hutt Alluvium	7.0	0.2	18.0	19.0	14.0	73.3	201

The **Pollution Load Index** (PLI) was calculated for the < 2mm sediment for each site following the method of Tomlinson *et al.* (1980) where:

$$PLI = (CF_1 \times CF_2 \times \ldots \times CF_n)^{1/n} \qquad \textit{Equation (2.3)}$$

where, n is the number of metals and **CF** is the **contamination factor**. The contamination factor is a ratio of the measured concentration to natural abundance of a given metal and is expressed as the following relation;

$$CF = \frac{metal\ concentration\ in\ the\ sediments}{background\ metal\ concentration}$$ *Equation (2.4)*

A PLI value >1 is considered polluted, while a PLI value <1 indicates no pollution. CF values are classified into four grades; <1 low contamination, 1 – 3 moderate contamination, 3 – 6 considerable contamination, and >6 very high contamination.

The **Potential Ecological Risk Index** (RI) is a measure of heavy metal pollution in the soil according to the toxicity of metals and the response of the environment proposed originally by Hakanson (1980). The RI is calculated using the following equations:

$$RI = \sum_{i=1}^{n} ER^i = \sum_{i=1}^{n} T^i \times CF^i$$ *Equation (2.5)*

Where, ER^i is the monomial potential ecological risk; T^i is the metal toxic response factor according to Hakanson. The toxic response factor values for each element are Zn = 1 < Cu = Pb = Ni = 5 < As = 10 < Cd = 30. There are four categories of RI; <150 low ecological risk, 150 – 300 moderate ecological risk, 300 – 600 considerable ecological risk, >600 very high ecological risk.

Statistical analysis

Statistical analysis was performed using the statistical software Rstudio Version 0.99.486 – © 2009-2015 RStudio, Inc. Pearsons correlation coefficients were calculated between trace elements for both the sediment and dissolved phase, %OM, physico-chemical parameters and proportion of impervious surface. A one-way ANOVA was performed to determine if there were any statistically significant differences between cities at $p<0.05$, if there was, then a post-hoc TukeyHSD test was performed to see where the difference lay. A principal component analysis (PCA) was used to reduce the high dimensionality of the variable space and was applied to the data of heavy metals. Heavy metal data were log transformed, scaled and centred to achieve comparable values between trace elements.

2.3 Results

The heavy metals reported in these reusults are those that exceeded guidelines at one or more site(s). These metals were As, Cu, Fe, Pb, and Zn for both water and sediment, Ag, Cd, and Ni in the sediment only, and Al and Cr for water only. The full dataset of heavy metal concentrations in both sediment and water can be found in Appendix 2. Analysis including sediment metal concentrations had three sites omitted (one in Auckland and two in Wellington) as sediment could not be collected at these sites.

No distinct differences in metal concentrations were observed between cities (Figure 2.1). The ordination explains 63.9% of the variation with axis PC1 effectively describing the variation in sediment metal concentrations (Table 2.5). All sediment metals aside from Ni, had strong (>0.60) and negative correlations with PC1. Therefore, the more negative the PC1 score, the more contaminated the sediments of that site were. Dissolved Al, Cu, Pb, and Cr had strong negative correlations with the axis PC2. Therefore, sites on the left of PC1 had high combinations of sediment metals. Those sites with more negative PC2 scores were likely to have greater water metal contamination, meaning sites towards the bottom of the ordination are likely to be high in dissolved Al, Cu, Pb, and Cr. The two PC axis were therefore used as a sediment (PC1) and water (PC2) metal index throughout this thesis for statistical analysis.

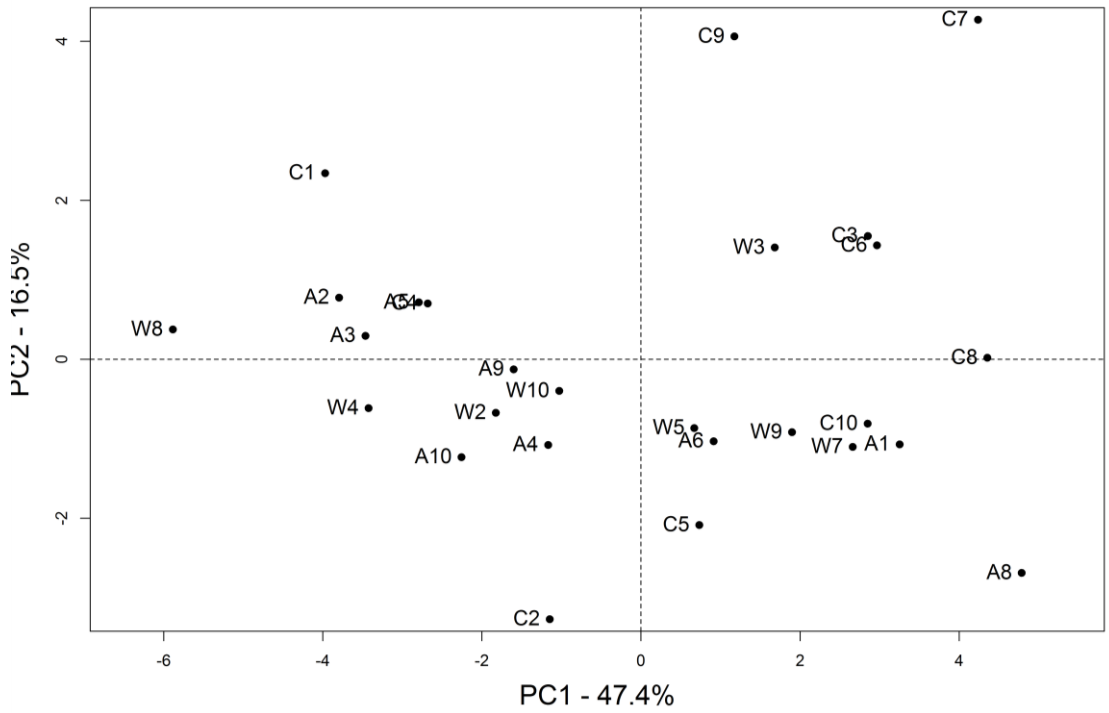

Figure 2.1: PCA of all heavy metals explaining 63.9% of variance in the data. A=Auckland, C=Christchurch, and W=Wellington.

Table 2.5: Principal component correlations for PC axis 1 and 2 with heavy metal concentrations. Those in bold indicate correlations higher than 0.60.

		PCA1	PCA2
<2mm sediment	**Fe**	**-0.73**	-0.02
	Ni	-0.56	-0.09
	Cu	**-0.90**	0.10
	Zn	**-0.90**	0.13
	As	**-0.84**	0.01
	Ag	**-0.65**	0.12
	Cd	**-0.90**	0.11
	Sb	**-0.81**	0.25
	Pb	**-0.77**	0.26
<63 µm sediment	**Cu**	**-0.82**	0.24
	Zn	**-0.84**	0.19
	As	**-0.80**	0.11
Water	**Al**	-0.24	**-0.68**
	Cu	-0.40	**-0.85**
	Zn	**-0.66**	-0.43
	As	-0.25	-0.54
	Pb	-0.40	**-0.65**
	Cr	0.00	**-0.77**

2.3.1 Individual heavy metals

The order of concentration was essentially the same between cities, dissolved metals for Auckland and Wellington were Zn>Al>Cu>As>Pb and for Christchurch Zn>Al>As>Cu>Pb. Similarly the concentration order for the < 2mm sediment was the same for Christchurch and Wellington with Fe>Zn>Pb>Cu>Ni>As>Ag and for Auckland Fe>Zn>Pb>Ni>Cu>As>Ag. The difference between the two was largely to do with the high concentrations of Ni in Auckland. The < 63µm sediment changes slightly with Zn, Cu and As showing significantly higher concentrations from the larger size fraction. Again Wellington and Christchurch were the same with Fe>Zn>Pb>Cu>As>N and Auckland Fe>Zn>Cu>Pb>Ni>As.

Table 2.6: Dissolved heavy metal mean, min, and max concentrations (µg L^{-1}) for the three cities Auckland, Christchurch, and Wellington (n=10).

		Al	As	Cr	Cu	Fe	Pb	Zn
	ANZECC 95% trigger	55.0	13.0	1.0	1.4	-	3.4	8.0
Auckland (n=10)	**mean ± 95% CI**	21.1 ±6.7	0.5 ±0.1	0.4 ±0.1	1.1 ±0.2	249.9 ±81.5	0.1 ±0	32.9 ±26.0
	min	5.6	0.2	0.1	0.6	47.6	<0.1	2.5
	max	41.5	0.7	0.7	1.6	436.0	0.2	141.1
	No. exceeding 95% ANZECC	0	0	0	2	-	0	7
Christchurch (n=10)	**mean ± 95% CI**	10.2 ±9.4	1.0 ±0.6	0.3 ±0.3	0.6 ±0.5	61.1 ±37.1	0.3 ±0.3	10.9 ±8.6
	min	1.0	0.1	<0.1	<0.1	10.5	<0.1	1.1
	max	50.1	2.7	1.4	2.9	166.9	1.6	44.4
	No. exceeding 95% ANZECC	**0**	0	1	1	0	0	3
Wellington (n=10)	**mean ± 95% CI**	15.0 ±6.0	0.6 ±0.1	0.2 ±0.1	1.3 ±0.4	148.4 ±93.3	0.1 ±0.1	25.3 ±23.2
	min	4.4	0.3	0.1	0.5	17.3	<0.1	1.7
	max	36.0	1.0	0.4	2.3	408.1	0.3	123.4
	No. exceeding 95% ANZECC	0	0	0	3	0	0	6

Table 2.7: Contamination factor (CF) means and 95% confidence intervals for the three cities Auckland (n=9), Christchurch (n=10), and Wellington (n=8).

	As	Cd	Cr	Cu	Ni	Pb	Zn
Auckland	0.40 ±0.08	0.24 ±0.10	0.26 ±0.06	0.45 ±0.15	0.57 ±0.34	0.70 ±0.38	0.50 ±0.36
Christchurch	0.47 ±0.34	0.63 ±0.28	0.65 ±0.11	0.69 ±0.23	0.54 ±0.09	0.90 ±0.38	1.66 ±1.17
Wellington	0.84 ±0.35	1.85 ±1.11	0.93 ±0.17	1.12 ±0.71	0.89 ±0.15	0.93 ±0.46	1.76 ±0.70

Table 2.8: Sediment heavy metal concentrations. Mean and 95% confidence interval, minimum and maximum concentrations (mg kg^{-1}) displayed for each city. < 63µm size fraction shown only for As, Cu and Zn as concentrations were significantly different.

		Ag	As	As	Cd	Cu	Cu	Fe	Ni	Pb	Zn	Zn
		< 2mm	**< 2mm**	**< 63µm**	**< 2mm**	**< 2mm**	**< 63µm**	**< 2mm**	**< 2mm**	**< 2mm**	**< 2mm**	**< 63µm**
	ANZECC ISQG-Low	1	20	20	1.5	65	65	-	21	50	200	
Auckland (n=9)	**mean ± 95% CI**	0.6 ±0.7	4.8 ±0.9	6.6 ±2.6	0.2 ±0.1	30.4 ±12.3	54.2 ±34.0	21145 ±4862	43.4 ±18.6	45.6 ±24.5	197.0 ±79.6	256.8 ±118.4
	min	BDL	2.3	2.1	BDL	6.3	6.9	7966	7.8	6.5	40.9	42.7
	max	2.8	6.6	15.5	0.3	61.5	159.2	28886	107.4	109.4	346.2	589.8
	No. exceeding ANZECC ISQG-Low	2	0	0	0	0	2	-	7	4	5	5
Christchurch (n=10)	**mean ± 95% CI**	0.1 ±0	5.7 ±3.8	7.8 ±5.7	0.1 ±0.1	14.1 ±4.9	22.5 ±5.3	13233 ±4143	8.7 ±1.5	45.7 ±13.3	238.1 ±167.3	377.2 ±303.4
	min	BDL	1.2	1.5	BDL	5.5	13.3	8073	6.1	17.3	43.5	62.9
	max	0.1	19.8	32.4	0.3	30.8	39.5	29885	13.4	86.1	869.2	1631.8
	No. exceeding ANZECC ISQG-Low	0	0	1	0	0	0	-	0	4	2	5
Wellington (n=8)	**mean ± 95% CI**	0.3 ±0.5	5.9 ±2.4	10.5 ±6.7	0.3 ±0.2	28.4 ±21.4	45.3 ±33.3	18503 ±2700	11.8 ±1.8	71.0 ±34.8	242.1 ±114.4	305.6 ±202.2
	min	0.1	2.9	2.7	0.1	8.5	9.9	13591	8.4	21.1	107.1	102.5
	max	1.9	12.9	26.2	0.7	102.7	139.7	246234	15.5	161.9	586.1	881.8
	No. exceeding ANZECC ISQG-Low	1	0	1	0	1	1	-	0	4	4	4

Metals exceeding ANZECC guidelines in water and sediment

Arsenic

Dissolved As concentrations ranged between 0.1 – 2.7 µg L^{-1} with average concentrations of 0.5, 1.0, and 0.3 µg L^{-1} for Auckland, Christchurch, and Wellington respectively (Table 2.6). On average, 97.2% of As was in the dissolved form and there was no significant difference in water arsenic concentrations between the three cities. No samples exceeded the ANZECC 95% trigger value, however, the ANZECC 99% trigger value of 1.0 µg L^{-1} was exceeded at five sites (four in Christchurch and one in Wellington). Dissolved As was significantly correlated with dissolved Pb and Cr (Table 2.10).

Sediment As concentrations ranged between 1.2 – 32.4 mg kg^{-1} for both the < 2mm and < 63µm size fractions across all three cities (Table 2.8). There was no significant difference between cities for sediment As concentrations. Concentrations in the <63 µm size fraction were significantly greater than the <2 mm (paired-t-test, t = -3.0, df = 25, p <0.01). No sites exceeded the ANZECC ISQG-Low trigger in the < 2mm sediment, however, two sites did in the < 63µm sediment. These were in Christchurch and Wellington at 32.4 and 26.2 mg kg^{-1} respectively. Arsenic in both size fractions significantly correlated with Cd, Cu, Fe, Pb, and Zn (p<0.01). Sediment As concentrations increased with %OM (< 2mm: r=0.51, p <0.01; < 63µm: r=0.44, p <0.05). Three sites were moderately contaminated with As based on the contamination factor (CF), two sites in Wellington, and one in Christchurch (Table 2.7).

Copper

Dissolved Cu ranged from below detection limits to 2.9 µg L^{-1} across all sites (Table 2.6). The average percentage of dissolved Cu was 81.7% compared to total Cu. Mean concentrations in Christchurch were significantly lower than in Wellington. Two sites in Auckland, one in Christchurch and three in Wellington exceeded the ANZECC 95% trigger value for Cu. Dissolved Cu was significantly correlated with dissolved Al and Zn (Table 2.10).

The sediment Cu concentrations were between 5.5 and 159.2 mg kg^{-1} across the three cities with no significant differences between the cities. However, there was a significant difference between the two size fractions (paired-t-test, t = -3.2, df = 25, p <0.01), with the < 63µm again generally having higher concentrations. Few sites exceeded the ANZECC ISQG-Low trigger value of 65 mg kg^{-1}, with only one site in the < 2mm sediment (Wellington) and three sites in the < 63µm (Wellington and Auckland). Sediment Cu concentrations were significantly correlated with sediment Ag, As, Cd, Fe, Ni, Pb, and Zn (see Table 2.10 for p-values). No correlation with %OM was observed. The CF identified three sites in Christchurch,

and two sites in Wellington as having moderate contamination compared to background concentrations (Table 2.7).

Lead

The majority of dissolved Pb concentrations were below detection limits with minimum concentrations all below 0.1 µg L^{-1} in each city (Table 2.6). The maximum concentration across the three cities was 1.6 µg L^{-1}, this site was in Christchurch. On average 42% of Pb was in the dissolved form. There were no significant differences between the three cities for dissolved Pb. While no sites exceeded the ANZECC 95% trigger value, one site in Christchurch did exceed the 99% trigger value of 1 µg L^{-1}. There was a significant relationship between dissolved Pb and dissolved As ($r=0.40$, $p<0.05$).

Sediment Pb concentrations ranged from 6.5 mg kg^{-1} to 161.9 mg kg^{-1} across the three cities (Table 2.8). There were no significant differences between the cities or between the two sediment size classes. Almost half of the sites exceed the ANZECC ISQG-Low trigger for Pb in sediment. Auckland, Christchurch and Wellington all had four sites each exceeding the ISQG-Low guideline. Sediment Pb was significantly correlated with sediment Ag, As, Cd, Cu and Zn (p-values in Table 2.10). The CF for each site found three sites in Auckland, five in Christchurch, and three in Wellington to have moderate contamination.

Zinc

Dissolved Zn concentrations ranged between 1.7 and 141.1 µg L^{-1} across the three cities and there were no significant difference between the cities. All three city averages were above the ANZECC 95% trigger value of 8 µg L^{-1}: Auckland at 32.9 µg L^{-1}, Christchurch 10.9 µg L^{-1}, and 25.3 µg L^{-1} for Wellington (Table 2.6). On average, 92% of Zn was present in the dissolved form. Over half of the sites exceed the 95% trigger value, seven in Auckland, three in Christchurch, and six in Wellington. Zinc is the only dissolved metal that significantly correlated with its associated sediment concentrations ($r=0.40$, $p<0.05$). Dissolved Zn also significantly correlated with dissolved Cu ($r=0.50$, $p<0.01$).

Concentrations of sediment Zn were between 40.9 and 1,632 mg kg^{-1} across the three cities (Table 2.8). There were no significant differences between the cities. The < 63µm size fraction had significantly greater Zn concentrations than the < 2mm size. All three city means in the < 63µm sediment exceeded the ANZECC ISQG-Low trigger, while two out of the three exceeded for the < 2mm sediment. There was a significant correlation between %OM and sediment Zn, with the < 63µm sediment showing a higher correlation ($r=0.54$, $p<0.01$). A number of significant correlations were presented for sediment Zn, including sediment As, Cd, Cu, Fe, and Pb. Moderate contamination was determined by the CF at ten sites (Table 2.7). This

included one site in Christchurch and Wellington which had considerable contamination and another site in Christchurch had very high contamination.

Metals exceeding ANZECC guidelines in water only

Aluminium

Dissolved Al concentrations across the three cities ranged between 1.0 and 50.1 µg L^{-1}, there were no significant differences between cities. Dissolved Al concentrations were 49% of total Al concentrations. The ANZECC 95% trigger value was not exceeded in any of the cities, however, five sites did exceed the 99% trigger value of 27 µg L^{-1} (Table 2.6). These sites were across the three cities, Auckland with three sites, and Christchurch and Wellington with one site each. There was a significant correlation between dissolved Al and dissolved Cu (r=0.40, p <0.05).

Chromium

Average concentrations of dissolved Cr were similar between cities (Table 2.6). The ANZECC 99% trigger value was lower than the detection limits for analysis (0.01 compared to the detection limit 0.1 µg L^{-1}), however, only six of the 30 sites were below detection limits. Therefore, the majority exceeded the ANZECC 99% trigger value. Christchurch had the only site exceeding the 95% trigger at 1 µg L^{-1}. There was not a large difference between total and dissolved Cr concentrations, with 91% of Cr in the dissolved form. Dissolved Cr significantly correlated with dissolved As (r=0.40, p <0.05).

Metals exceeding ANZECC guidelines in sediment only

Cadmium

Sediment Cd concentrations were all below the ANZECC ISQG-Low trigger value (Table 2.8). However, when comparing to background concentrations and calculating the CF, two sites in Christchurch and three in Wellington had moderate contamination (1.3, 1.6 and 1.9, 1.1, 1.7). Another two sites in Wellington also had considerable contamination. There was no correlation with %OM. Significant correlations were observed for sediment Ag, As, Cu, Pb, and Zn (Table 2.10).

Nickel

The Auckland average sediment Ni concentration was 43.4 mg kg^{-1} which is above the ANZECC ISQG-Low trigger of 21 mg kg^{-1}. Neither Christchurch or Wellington had sites that exceeded this trigger value. Sediment Ni concentrations were significantly different between Auckland and both Christchurch and Wellington. There was no significant difference between size fractions. There were significant correlations

between sediment Ni and Ag, Cu, and Fe (p-values in Table 2.10). Auckland had two sites and Wellington three sites with moderate contamination according to the CF (Table 2.7).

Silver

Silver concentrations in the sediment ranged from below detection limits to 2.8 mg kg^{-1}. There were no significant differences between the cities. The majority of the sites were well below the ISQG-low trigger (Table 2.8). Three sites exceeded the trigger, two in Auckland and one in Wellington. There were significant correlations with sediment Cd, Cu, Ni, and Pb. Background values for sediment Ag were not available, thus the CF could not be determined.

2.3.2 Pollution indices

Cumulative criterion unit

The CCU was calculated for each site in the three cities. There was no significant difference between the mean CCUs for any of the cities, this was primarily due to the high variation in CCUs between the 10 sites in each city (Figure 2.2a). Low metal contamination was present at four sites in Auckland. The majority of sites in Christchurch had very low CCU values while two sites in Christchurch had intermediate levels of contamination, as classified by the CCU categories (Figure 2.2b). Low metal contamination was at two Wellington sites and one site had intermediate levels of contamination.

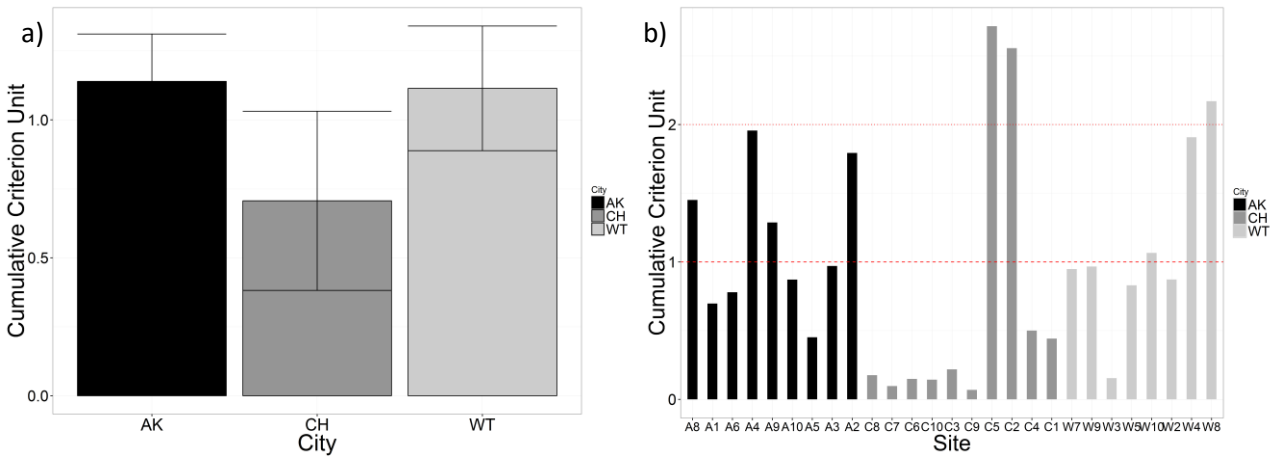

Figure 2.2: CCU for Auckland, Christchurch and Wellington a) city means error bars indicate ± 1 S.E. b) CCU of individual sites, dashed line indicates threshold for low metal contamination, dotted line indicates threshold for intermediate levels of contamination. Sites ordered by PCA sediment metal index.

Sediment pollution indices

There was a significant difference (TukeyHSD, PLI: p <0.05; RI: p <0.01) between Auckland and Wellington for both sediment pollution indices, with Wellington values being higher than that of Auckland (Figure 2.3). Wellington was significantly higher than Christchurch (TukeyHSD, p <0.05) for the RI (Figure 2.3c). Only five sites were classifies to be polluted according to the PLI, where values >1 are polluted. These sites were in Christchurch (three) and Wellington (two). The Risk Index had two sites in Wellington with low risk to ecological health and no other sites of ecological risk in terms of metal pollution.

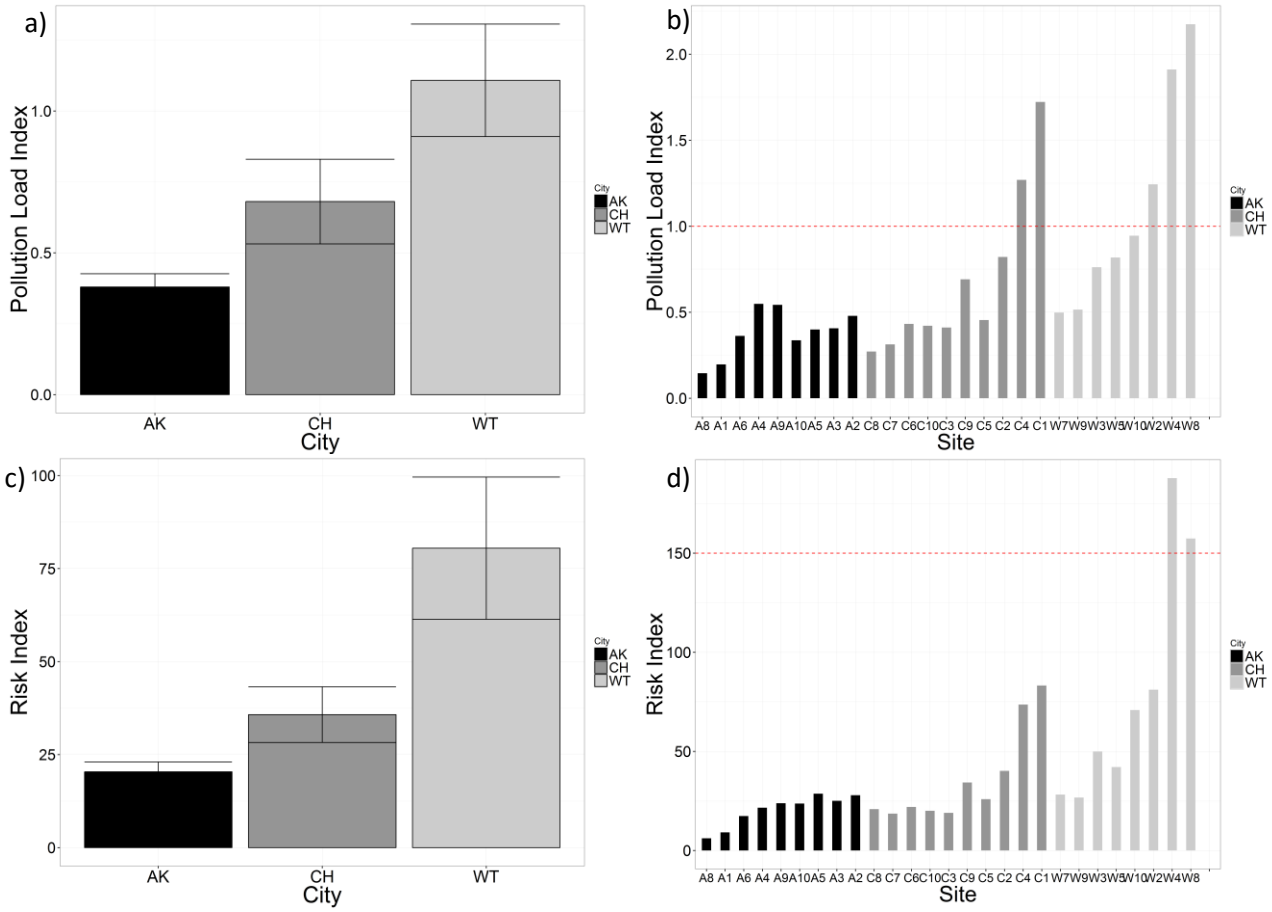

Figure 2.3: Pollution indices a) City means for Pollution Load Index (PLI) and error bars indicating ± 1 S.E. b) PLI for individual sites, sites are ordered by the sediment (PC1) metal index within each city, dashed line represents threshold for contamination c) City means for Risk Index (RI) with error bars indicating ± 1 S.E. d) RI of individual sites ordered within each city by sediment (PC1) metal index. Dashed line indicates threshold for low ecological risk.

2.3.3 Impervious area

While catchment land use was predominantly urban for all sites, the surrounding land use comprised of largely residential with over half of the sites being located in or adjacent to parks or reserves (Appendix 1 Table 6.2). The impervious surface area for each site was above 10% and reached up to 96% in Christchurch (Figure 2.4).

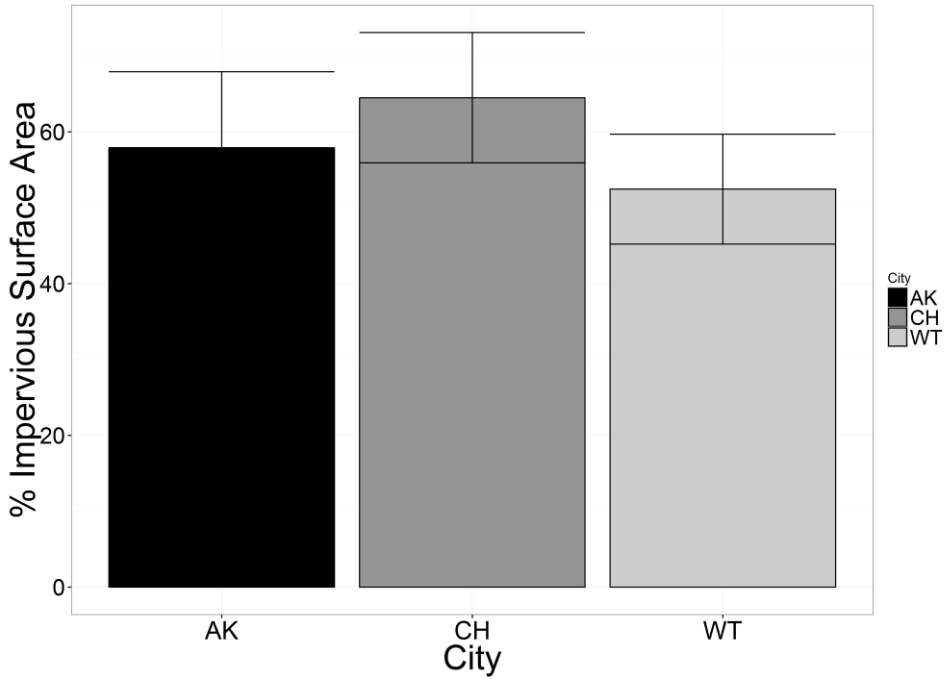

Figure 2.4: Percent impervious surface area means for the three cities Auckland, Christchurch, and Wellington (n=10). Error bars are ± 1 S.E.

Dissolved As, Pb, and Zn concentrations increased with impervious area (p <0.05). For sediment, As, Cd, Pb, and Zn concentrations in the < 2mm sediment also increased with increasing impervious area (p <0.05). Zinc was the only heavy metal in the < 63µm sediment for which there was a significant positive correlation with impervious surface area (Table 2.9). Overall As, Cd, Pb, and Zn concentrations generally increased with increased impervious surface area, whereas the other metals did not.

Table 2.9: Pearson correlation factors (r) between % impervious surface area and individual heavy metals.

Dissolved Water (n=30)	r	< 2mm Sediment (n=27)	r	< 63µm Sediment (n=26)	r
Al	-0.21	**Ag**	0.19	**Cu**	0.19
As	0.44*	**As**	0.38*	**As**	0.36
Cr	0.11	**Cd**	0.43*	**Zn**	0.53**
Cu	0.34	**Cu**	0.23		
Pb	0.39*	**Fe**	0.1		
Zn	0.42*	**Ni**	0.01		
		Pb	0.40*		
		Zn	0.55**		

Stars denote significance: *p<0.05, **p<0.01

To investigate the relationship between impervious area and all the heavy metals combined, PC1 from the PCA performed was used as a composite 'metal index'. This axis accounted for 47% of the variation and effectively ranked the sites on metal contamination (Figure 2.1). There was a significant negative correlation between this index and the proportion of impervious area ($r^2 = 0.189$, $p < 0.01$). More negative metal index values corresponded to greater overall concentrations of heavy metals (Figure 2.5). Therefore, as the impervious area increased, so too did the overall heavy metal concentrations.

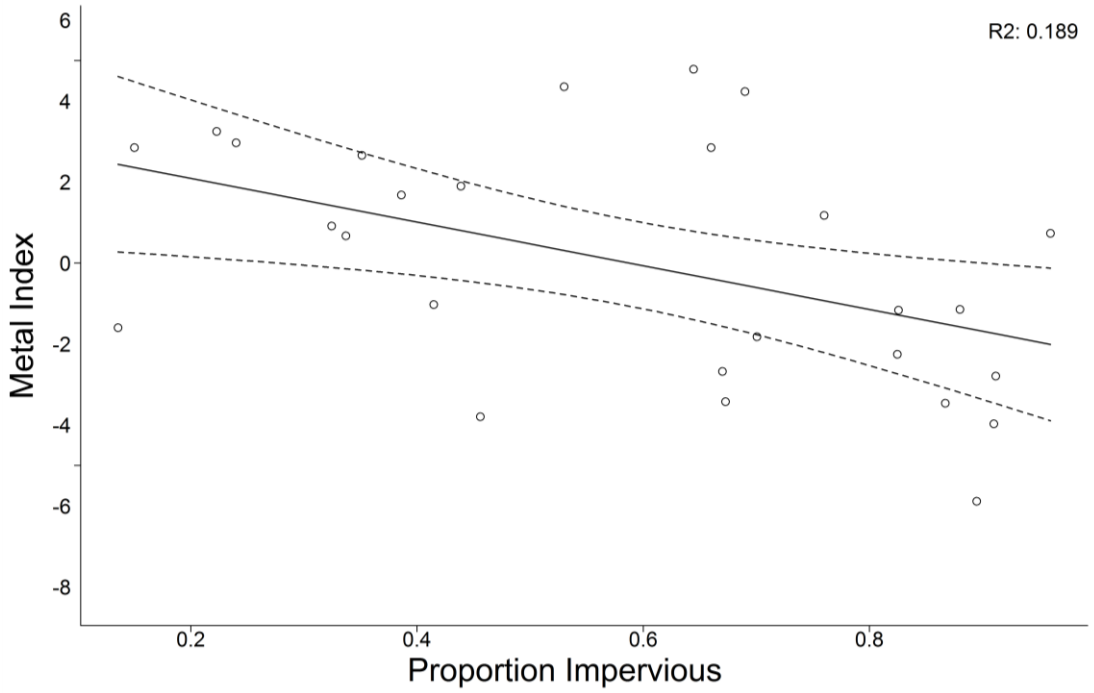

Figure 2.5: Linear regression between the composite metal index and proportion of impervious surface area. Dashed lines represent 95% confidence intervals.

Table 2.10: Pearsons correlations between heavy metals in sediment and water, and %OM. Those in bold are statistically significant, those highlighted also have r>0.7

			< 2mm sediment								Water						< 63µm sediment	
		%OM	Fe	Ni	Cu	Zn	As	Ag	Cd	Pb	Al	Cu	Zn	As	Pb	Cr	Cu	Zn
< 2mm sediment	Fe	0.33																
	Ni	0.06	**0.61****															
	Cu	0.24	**0.56****	**0.63****														
	Zn	**0.48***	**0.52****	0.1	**0.52****													
	As	**0.51****	**0.60****	0.03	**0.42***	**0.87****												
	Ag	0.11	0.18	**0.63****	**0.77****	0.3	0.09											
	Cd	0.18	0.37	0.13	**0.74****	**0.68****	**0.63****	**0.49****										
	Pb	0.13	0.3	0.16	**0.74****	**0.66****	**0.54****	**0.58****	**0.84****									
Water	Al	0.17	0.21	0.17	0.04	-0.12	0.08	-0.04	-0.15	-0.27								
	Cu	-0.08	0.21	0	0.19	0.14	0.17	0.01	0.36	0.34	**0.40***							
	Zn	0.01	**0.42***	0.34	**0.52****	**0.40***	0.24	**0.41***	**0.54****	**0.42***	-0.06	**0.50****						
	As	0.04	-0.2	-0.24	-0.16	0.11	0.07	-0.1	0.09	0.02	0.04	0.33	0.11					
	Pb	-0.15	-0.19	-0.14	-0.07	0	-0.04	-0.03	0.06	0.02	0.09	0.26	0.15	**0.76****				
	Cr	0.3	-0.27	-0.04	-0.16	-0.27	-0.24	-0.02	-0.11	-0.19	0.05	0.13	-0.13	**0.40***	0			
< 63µm sediment	Cu	0.17	**0.46***	**0.38***	**0.80****	0.38	0.32	0.35	**0.62****	**0.56****	0	0.17	**0.40***	-0.11	-0.03	-0.16		
	Zn	**0.54****	**0.47***	0.07	**0.46***	**0.96****	**0.85****	0.22	**0.57****	**0.55****	-0.09	0	0.27	0.13	0.03	-0.26	0.38	
	As	**0.44***	**0.50****	-0.02	**0.54****	**0.77****	**0.86****	0.17	**0.69****	**0.60****	0.06	0.18	0.28	0.06	0.03	-0.22	**0.54****	**0.80****

*p <0.05, **p <0.01

2.4 Discussion

Generally, all three cities had sites with high and low metal concentrations. As a result, there was little overall difference between cities for individual metal concentrations. However, dissolved Cu and Ni in the sediment were significantly different between Wellington and Christchurch, and Auckland to Wellington and Christchurch respectively. This was also confirmed by the PCA, where there were no significant groupings of each city. Although, there did appear to be slight grouping of Wellington and Auckland towards the middle of the PC2 axis, whereas many Christchurch sites are at the extremes of the PC2 axis. Given that PC2 is largely influenced by dissolved Cu, Cr, and Pb, this suggests that the dissolved heavy metal concentrations are highly variable in Christchurch. This is in fact the case, with five out of the seven dissolved heavy metals that exceeded guidelines having the largest variability in Christchurch.

The pollution indices combine the heavy metals based on either toxicity or comparison to background concentrations of each metal, to produce values that indicate the combined toxicity or contamination. The Cumulative Criterion Unit (CCU) for Christchurch reflected the results of the PCA. The two sites with the highest contamination in the dataset were in Christchurch and the remaining sites in Christchurch were well below the trigger for low metal contamination. It is surprising that many of the CCU's are below the toxic threshold (<1) considering literature has found less urbanised catchments in other countries to consistently exceed the low metal contamination threshold (<1) (Robson *et al.* 2006).

The sediment pollution indices (PLI and RI) indicate that contamination is low when compared to background concentrations, as many of the values were less than one. In contrast, many sites and heavy metals exceeded the ANZECC ISQG-Low trigger. For Auckland, this is largely due to approximately half the sites being volcanic (ARC 2001) and thus background concentrations of many metals are naturally elevated. The relatively few sites showing contamination compared to the number of sites exceeding ANZECC guidelines may also be a result of using the maximum value from the range of background concentrations given in ARC (2001), Sulzberger and Whitty (2003), and Tonkin & Taylor (2007). These maximum concentrations may have been affected by unknown anthropogenic activities. For example, the Auckland Regional Council (ARC 2001) reported, soils that "had not been significantly disturbed for a significant period" (50 years) were used. Therefore, these sites may still have had some sort of anthropogenic input. Furthermore, although background soil samples were considered separately for each geologically different area, the maximum value may be localized to smaller scales and not relevant to the particular sites sampled in this study, due to the large range of some heavy metals. However, the ANZECC Guidelines for Assessment and Management of

Contaminated Sites recommend the evaluation of background levels as a consideration in remediation efforts (ANZECC 1992). Although the background concentrations may be over-estimated at some sites, it is important to consider them in order to maximise monitoring and remediation efforts.

The three most common heavy metals identified in urban systems are Cu, Pb, and Zn (Davis *et al.* 2001; Walsh *et al.* 2005). These heavy metals are discharged as stormwater to waterways from anthropogenic sources such as galvanised roofs and road runoff (Davis 2010). Zinc is certainly the most prominent heavy metal from anthropogenic sources in this study. It was the most common metal to exceed guidelines for water and sediment in all three cities. Only two sites did not exceed the ANZECC 99% trigger value in dissolved water samples. This demonstrates that even sites that have low contamination overall, have elevated levels of dissolved Zn. It has previously been reported that Zn concentrations increase at the greatest rate with impervious area (Pettigrove and Hoffmann 2003). Zinc is also a main constituent of roof runoff, and brake and tyre wear (Davis *et al.* 2001; Herald 2003), therefore it is not surprising that Zn concentrations are consistently elevated. There is limited literature on heavy metal concentrations in urban streams in New Zealand outside of council data. The latest council reports for each corresponding city (Heath *et al.* 2014; Lockie 2014; Margetts and Marshall 2015) largely agree with this study in that the majority of urban sites exceed the ANZECC 99% trigger value for Zn.

Lead was below detection limits at the majority of sites for water samples, however, almost half the sites exceed the ISQG-Low trigger value. The higher concentrations of Pb in the sediment are likely to be a legacy effect of the use of leaded petrol (Albanese and Breward 2011). However, even with the banning of leaded petrol, studies have still found elevated concentrations of lead in road dust samples (Duong and Lee 2011). Lead can still be sourced from brake and tyre wear and lubricating oils (Davis *et al.* 2001; Beasley and Kneale 2002; Duong and Lee 2011) and could still be entering freshwater systems as particulate matter. Lead is predominantly in the particulate form in this study. While peak Pb concentrations have decreased, there are still sources that may have contributed to elevated sediment concentrations.

Copper, exceeded the ISQG-Low trigger at only a few sites but multiple sites exceeded the ANZECC 99% trigger value. The mean sediment Cu concentrations for each city were reasonably low compared to studies in other urban areas internationally, which generally would exceed the ANZECC ISQG-low trigger of 65 mg kg^{-1} (Hnatukova *et al.* 2009; Howard and Olulu 2013; Sutherland 2000). Copper largely comes from brake pad wear making it a common metal in road runoff (Davis *et al.* 2001). It was also the only other metal which exceeded the ANZECC 95% trigger value for dissolved Cu at more than one site in this study. Dissolved Cu was the only heavy metal in the water samples for which there were significant differences between cities. Christchurch concentrations were all well below the 99% trigger

value, apart from one site that exceeded the ANZECC 80% trigger. This agrees with data obtained from councils, where dissolved Cu is largely below detection limits in Christchurch (Margetts and Marshall 2015) and median concentrations for Auckland and Wellington consistently exceeded guidelines (Lockie 2014; Heath *et al.* 2014). The reason for this difference is unclear and could warrant further investigation.

Chromium is also associated with brake pads and tyres (Paul and Meyer 2008), however, it is not often identified as a metal of concern in urban streams. The ANZECC 99% trigger value (0.01 µg L^{-1}) is below the detection limit of 0.1 µg L^{-1} for this study. However, all but six sites were above detection limits meaning that the majority of sites may exceed the guideline. Although only one site exceeded the 95% trigger, it may be a metal that requires continued monitoring to ensure it does not increase any further.

Comparing sediment metal concentrations to background concentrations is recommended by the ANZECC committee before determining remediation efforts. Both cadmium and nickel provided fitting examples of this in this study. Nickel sediment concentrations in Auckland appear to be rather high with seven sites exceeding the ISQG-Low guideline. However, the isthmus volcanic field in the Auckland region has naturally high levels of Ni (ARC 2001), and once accounting for this with the contamination factor, only two sites in Auckland presented low metal contamination.

Cadmium however, had concentrations well below ANZECC guidelines indicating that there were no elevated concentrations at the investigated sites. In contrast, cadmium is often reported as a common metal in urban environments (Paul and Meyer 2008). Calculation of the contamination factor however, resulted in five sites showing low metal contamination and a further two at intermediate levels of contamination. While the concentrations presented may not pose risk to aquatic life, it indicated that contamination of Cd is occurring and as for Cr, it may be a metal to continue monitoring.

Correlations between heavy metals can provide interesting information on sources and pathways. The majority of sediment metals had significant correlations, however, few were very strong. Lead, cadmium, and copper had Pearson correlations greater than 0.7 (r^2> 0.49) with each other, suggesting perhaps a common source. These three metals are not reported to have a common source, however it may be that the correlation relates to their occurrence in road dust (Davis *et al.* 2001). Correlations in other studies have shown similar results, Manta *et al.* (2002) found strong correlations for Pb/Zn and Cu/Sb. Sekabira *et al.* (2010) also found significant correlations for Pb with Zn and Cu (Sb was not measured). Sutherland (2000) reported correlations for Pb with Cd, Cu, Zn and Fe. The three main stormwater heavy metals Cu, Pb and Zn appear to consistently correlate with each other across

studies. Although not a strong correlation (r<0.7), Zn did significantly correlate with Cu and Pb in the current study.

The strongest correlation in this study was between sediment Zn and As (r=0.87). Kalender and Çiçek Uçar (2013) also found a significant positive correlation between Zn and As, along with a number of others. Correlations between dissolved metals were limited, the significant relationships being between Cu/Al, Cu/Zn, and As/Pb. The As and Pb correlation was the only one to be above 0.7, while the others were relatively weak.

In this study, there was a significant correlation between sediment and dissolved Zn, however, it was relatively weak (r=0.40). No other heavy metals had significant correlations between sediment and water concentrations. Widianarko *et al.* (2000) also found no association between water heavy metals and the associated sediment concentrations for Cu, Pb, and Zn. This highlights the importance for monitoring both sediment and water for heavy metals. Many councils routinely monitor only the water phase, however, some literature suggest that sediment is more descriptive of the heavy metal contamination and should be used over water as a monitoring tool (Chakravarty and Patgiri 2009). Sediment metal concentrations can be used as a sample integrated over time and eliminates the problem often observed with water samples of fluctuations in metal concentrations (Kiffney and Clements 1994). Such fluctuations may include peak concentrations of metals that are in fact toxic to aquatic life, however these events are rarely detected through routine sampling (Perdikaki and Mason 1999).

There are many other factors aside from anthropogenic and lithogenic sources that determine the concentration in sediments. For example, it is important to investigate smaller size fractions as it has been commonly reported that pollutant metal concentrations increase as the sediment particle size decreases (Zanders 2005). Higher concentrations were found in the < 63µm sediment for Zn, Cu and As in this study. This would mean that there is a potential to not understand the true level of contamination if only the larger size fraction is investigated.

Another factor is the strong affinity between metals and organic matter (Lin and Chen 1998). In theory, a greater percentage of organic matter would result in higher concentrations of metals. Significant correlations with %OM were only found for Zn and As in this study. These correlations were also relatively weak. Paul and Meyer (2001) summarised that both bed and suspended sediments with high organic matter content frequently exhibit 50 -7500 times higher concentrations of metals than sediments with lower organic matter content. The loss on ignition results from this study indicated relatively low organic matter, the highest percentage being 15%. This may explain the lack of relationship between %OM and heavy metals. Significant correlations with Fe were observed,

although again, not very strong, all being less than 0.7. This indicates some affinity to Fe oxides for metals As, Cu, Ni, and Zn.

As impervious area is commonly reported as being an accurate predictor of urbanisation (Morse *et al.* 2003; Allan 2004), it would be expected that the relationships between percent impervious and anthropogenic heavy metals would be relatively strong and significant. Total catchment imperviousness and Zn have been shown to have a strong relationship previously (r >0.9), however this is not always consistent (Walsh 2004). Significant correlations were found in this study between impervious surface area and As, Cd, Pb, and Zn concentrations in both the sediment and water phases. However, these Pearson correlations were all less than 0.55 (r^2<0.30). The metal index from the PCA was similar, with a significant correlation of r^2=0.20. It may be that drainage infrastructure provides a more significant predictor than impervious surface area, as it is also considered a primary determinant of the quality of urban stormwater runoff delivered to receiving waterways (Walsh 2000).

2.5 Conclusions

This study is the only known study to compare metal concentrations in urban streams in different cities in New Zealand. There were generally no significant differences in metal contamination between the three cities; Auckland, Christchurch, and Wellington. The exception to this was dissolved Cu, which was significantly lower in Christchurch, and sediment Ni, which was significantly higher in Auckland due to the volcanic soil.

As expected, Cu, Pb, and Zn were the most prominent metals and exceeded ANZECC guidelines frequently. However, Cr and Cd may be metals of increasing concern in the urban environment. Sediment metals generally increased together as demonstrated by the PCA, with the strongest correlations being between Pb, Cd, and Cu. A high dissolved metal concentration did not necessarily mean high concentrations of that metal in the sediment. This was exemplified by the lack of correlations between sediment and dissolved metals. The < 63µm sediment had significantly higher concentrations for As, Cu, and Zn than in the < 2mm sediment.

3 Chapter 3: The response of stream invertebrate communities to metals in three cities

3.1 Introduction

Benthic invertebrate communities in urban streams have been shown to decrease in complexity and to be comprised of pollution tolerant taxa such as Oligochaeta (Walsh 2000; Morse *et al.* 2003). This change in community composition arises at very low levels of urbanisation, as low as 6% impervious surface area (Klein 1979; Morse *et al.* 2003). There are many factors that contribute to the degradation of stream ecosystems, known as the 'urban stream syndrome' (Chapter 1). While these factors have been consistently identified as responsible for degrading urban streams, it is not clear as to the relative contributions of each factors. Stormwater contaminants such as heavy metals have been identified as key causes of reductions in sensitive benthic invertebrate abundance and stream health (Beasley and Kneale 2002). However, determining the mechanisms by whichthose contribute to the changes in benthic invertebrate communities is challenging.

Benthic invertebrate surveys of urban streams in New Zealand are largely limited to council monitoring programmes. While large scale studies are few, there are studies on individual catchments or streams in urban areas (Blakely and Harding 2005). The Auckland Council has a River Ecology Programme for a number of streams in the greater Auckland region, however, there is not yet a regular reporting structure for this (L. Buckthought 2016, pers. comm.). Raw data was provided from the Auckland Council on benthic invertebrate species in urban streams, suggests sites have generally 'poor' or 'fair' water quality based on biotic indices.

The Christchurch City Council conducts monitoring of benthic invertebrates for the five catchments in Christchurch on a five-yearly cycle, sampling one catchment each year (McMurtrie 2009). The latest report for the Avon catchment (the most urbanised catchment) had the majority of sites classed as 'poor' based on biotic indices, with no taxa from Ephemeroptera or Plecoptera orders (Blakely 2014).

The State of the Environment monitoring conducted by the Greater Wellington Regional Council includes only seven sites that are considered to be in urban catchments (Heath *et al.* 2014). Their monitoring includes annual benthic invertebrate sampling. The latest report state these seven sites are generally degraded, and classed mostly as 'poor' and 'fair' water quality based on biotic indices (Heath *et al.* 2014).

Overall, there have not been many studies on benthic invertebrate communities of urban streams (Blakely and Harding 2005), and no studies could be found comparing multiple cities within New

Zealand. In addition, the monitoring of urban streams by councils often perform benthic invertebrate sampling and analysis of heavy metals at different times, making determination of relationships between the two difficult. The aims of this study were to:

- Determine the ecological health of urban streams around Auckland, Christchurch, and Wellington based on benthic invertebrate commonities.
- Determine if there is a relationship between physical characteristics of the catchments and the benthic invertebrate community.
- Determine if there were significant relationships between invertebrates and heavy metal concentrations

3.2 Methods

3.2.1 Physico-chemical characteristics

Methods for physico-chemical characteristics were described in Chapter 2 (section 2.2.3). Basic water chemistry parameters of pH, dissolved oxygen, temperature, and specific conductivity were measured at each site. Depth and wetted width was noted along with surface velocity and habitat assessments and a substrate index (SI) was calculated for each site.

3.2.2 Macroinvertebrate sampling

The benthic invertebrate and metal sampling occurred at the same time in late autumn (Christchurch – April, Wellington – May, Auckland – June 2015). Benthic Invertebrate samples were collected as a single composite kick-net sample (500 µm) at each site. The kick-net sample was used to determine species diversity. Composite samples were collected from approximately five kicks covering a range of habitats within the reach (approximately 20 m) at each site (e.g. riffle, pool, leaf and wood packs, slow water zones). Substrate was vigorously disturbed in front of the kick-net allowing material to be caught following the sampling protocols by Stark et al (2001). Samples were stored in plastic containers and preserved with 70% ethanol and returned to the laboratory for analysis.

In the laboratory, Protocol P3 (Stark et al 2001) was used to sort the macroinvertebrates to lowest practical taxonomic level using Winterbourn *et al.* (2006) and Winterbourn (1973). Benthic samples were rinsed through a 500 µm sieve and placed into a white tray to pick out invertebrates. Most insect taxa were identified to genus level, while generally non-insect taxa were identified to class or order.

3.2.3 Data analysis

Taxonomic richness, number of EPT (Ephemeroptera, Plecoptera, and Trichoptera) taxa, the Macroinvertebrate Community Index (MCI) (Stark 1985), and the Urban Community Index (UCI) (Suren

et al. 1998) were determined for each site. The hydroptilid caddisfly, *Oxyethira* spp. was excluded from the EPT taxa as they are often found in degraded waters (Death and Collier 2010).

The MCI is a biotic index used to measure organic enrichment in stony riffles using tolerance scores. It is calculated from presence-absence data as follows:

$$\text{MCI} = \frac{\sum_{i-1}^{i-S} a_i}{S} \times 20 \qquad \textit{Equation 3.1}$$

where *S* = the total number of taxa in the sample, and a_i is the tolerance value for the *i*th taxon (Appendix 3)

MCI values <80 are considered poor, 80-99 are fair, 100-119 are good, and >119 are excellent.

The UCI is an index created by Suren *et al*. (1998) that can be used to determine the health of urban and periurban streams. It was specifically developed for New Zealand urban streams and based on a multivariate analysis of urban streams throughout the country. It is calculated in a similar way to the MCI with tolerance scores for invertebrates and based on presence-absence data. Some taxa found in the study did not have assigned tolerance scores, therefore, tolerance scores of taxa that were of the same family or of similar sensitivity were used.

$$UCI = \sum_{i=1}^{i=S} \frac{a_i}{s} \times 20 \qquad \textit{Equation 3.2}$$

where *S* = the total number of taxa in the sample, and a_i is the tolerance value for the *i*th taxon (Appendix 3)

Negative scores are indicative of pollution tolerant invertebrate communities and silted habitats. Positive scores are indicative of invertebrate communities found in fast-flowing, cobble streams with few macrophytes.

Statistics

The sediment metal index (chapter 2) did not include one site in Auckland and two in Wellington as sediment samples could not be obtained at these sites. Therefore, those sites were excluded in the following analysis.

Invertebrate communities between sites were assessed using presence/absence data and non-metric multidimensional scaling (NMDS). Analyses were based on Bray-Curtis dissimilarities between samples. Ordination scores, or sites, that are close together on the resulting plot are more similar in benthic invertebrate community composition than those further apart. The 'goodness of fit' is determined by the stress value. A stress value of 0 indicates a perfect fit, a value up to 0.20 is considered acceptable. The stress value for this 2-dimensional solution was 0.20. Analysis of similarity

(ANOSIM) was used to determine if benthic invertebrate communities differed between cities. The ANOSIM statistic R varies from 0 (no difference among groups) to 1 (groups are completely distinct).

A Canonical Correspondence Analysis (CCA) (from the Vegan package in R) was performed on the benthic invertebrate community data with environmental variables that were identified as significant from the envfit function on the NMDS. The environmental variables constrain the ordination, therefore, the ordination does not explain all the variance in the dataset, only the part that can be explained by the constraints (i.e. the environmental variables). Sites or benthic invertebrate genera in the direction of the vector arrow will be strongly positively correlated with and influenced by the environmental variable. Those sites or invertebrate genera whose projections lie near the origin will be less strongly affected.

One-way analysis of variance (ANOVA) was used to determine any differences between the means of the biotic indices for the three cities. The models were checked for normality and homogeneity of variance of the residuals and the significance level was set at 0.05.

Step-wise model selection in the R package MASS was used to investigate significant predictor variables based on the AIC of the model. Multiple linear regression was used for the MCI and UCI indices. However, as taxonomic richness and EPT taxa were count data, generalised linear models (GLMs) were used with a poisson distribution. A 'goodness of fit' measure for the GLMs were determined as a pseudo-r^2 calculated as:

$$Pseudo - r^2 = \frac{null\ deviance - residual\ deviance}{null\ deviance} \times 100 \qquad \textit{Equation 3.3}$$

All significance levels were determined to be p <0.05.

3.3 Results

3.3.1 Catchment and surrounding land use

See section 2.3.3

3.3.2 Physical habitat conditions

The 30 sites across the three cities varied substantially in physical condition. Some sites had relatively natural channels with riffle, run, pool complexes where others were highly channelized and reduced to concrete drains (Figure 3.1). No sites in Wellington were concreted, however, two sites had culverts. Sites ranged from 0.8 and 6.2 m wetted width and had similar average depths between cities. Stream bed substrate size varied markedly with Auckland having the largest range with a concrete site resulting in a SI value of zero (Table 3.1).

ANZECC guidelines recommend pH should be within the range of 6.5 – 8.5 and DO to be above 6 mg L^{-1}. Auckland had sites that were outside the range for pH, with two sites <6.5 and one >8.5. Both Christchurch and Wellington had one site each that was below the DO concentration. Water velocities were also highly variable ranging from 0 to 0.73 ms^{-1}. While specific conductivity was lowest in Christchurch (106 μS_{25} cm^{-1}) and highest in Auckland (905 μS_{25} cm^{-1}) which was likely to be a salinity influence (salinity was 0.4 whereas all other sites in Auckland were 0.1). The temperature range did not exceed the proposed recommended upper limit of 24°C (Davies-Colley *et al.* 2013).

Figure 3.1: Photographs of selected sites representing the range of sites sampled. Photos a) – c) show sites with riffle, run, pool complexes and riparian vegetation, d) – f) show highly channelised and artificial banks.

Macrophytes commonly occurred in urban streams, being present in 76.7% of sites (23 sites). Canopy cover varied between and within cities and classes of "open", "partial", and "heavily shaded" were distributed relatively evenly across the sites and the three cities. A summary table of habitat at each site is presented in Appendix 1.

Table 3.1:Physical and chemical parameters for the 30 sites sampled in three cities between March and June 2015. Values are presented as ranges with mean in brackets. n=10 sites per city and data is based on a single sampling occasion. SI = substrate index.

	SI	Depth (m)	Width (m)	Velocity ($m\ s^{-1}$)	pH	Specific Conductivity ($\mu S_{25}\ cm^{-1}$)	Temperature (°C)	DO ($mg\ L^{-1}$)
Auckland	0.0 – 5.6 (3.2)	0.02 - 0.40 (0.16)	0.80 - 3.20 (2.02)	0.02 - 0.73 (0.23)	6.1 - 9.4	174 - 905 (310)	8.9 - 16.3 (12.6)	6.6 - 7.6 (7.5)
Christchurch	2.0 – 5.1 (4.3)	0.04 - 0.32 (0.16)	1.00 - 5.00 (2.53)	0 - 0.48 (0.23)	6.5 - 7.4	106 - 270 (176)	9.4 - 15.9 (13.6)	5.1 – 10.0 (7.5)
Wellington	4.7 – 5.6 (5.2)	0.04 - 0.21 (0.10)	1.00 - 6.20 (2.90)	0.10 - 0.69 (0.36)	6.9 - 8.2	193 - 325 (241)	7.1 - 10.1 (8.8)	4.9 - 12.0 (10.8)

3.3.3 Benthic invertebrate diversity and communities:

A total of 49 invertebrate taxa were collected across the 30 sites, of these the highest diversity was found in Wellington (37) and the lowest in Christchurch (25). The most diverse order across the cities were the Trichoptera, with 14 taxa. This was followed by Diptera with seven taxa, Ephemeroptera and Plecoptera with five taxa, and Crustacea and Molluscs with four taxa each.

The most commonly occurring taxa were Oligochaeta and the hydrobid snail *Potamopyrgus* spp. (both occurring at 27 sites) (Table 3.2). Auckland and Christchurch were similar with Oligochaeta, and the snails *Potamopyrgus* spp. and *Physa* sp. (now *Physella*) present at the most sites. In contrast, Wellington streams were dominated by Collembola which occurred at nine sites (Table 3.2). A full list of taxa at each sites is presented in Appendix 3.

Table 3.2: Five most common benthic invertebrate taxa in each city of Auckland, Christchurch, and Wellington. The number of sites each species occurs are in parentheses.

Auckland	Christchurch	Wellington
Oligochaeta (9)	Oligochaeta (10)	Collembola (9)
Potamopyrgus spp. (9)	*Potamopyrgus* spp. (10)	*Potamopyrgus* spp. (8)
Physa sp. (7)	*Physa* sp. (10)	Amphipoda (8)
Cura sp. (6)	Ostracoda (9)	Ortholcad (8)
Amphipoda (5)	*Sphaerium* sp. (9)	Oligochaeta (8)

Distinct differences in invertebrate communities between Wellington streams and the other two cities are apparent in the NMDS (Figure 3.2). Wellington sites generally had higher NMDS1 axis scores. In contrast, Christchurch sites had a sub-set of fauna found within Auckland. Importantly, Christchurch streams had communities which were more similar to each other than Auckland streams which had highly variable communities. A significant difference between the city groups was confirmed by ANOSIM ($R=0.41$, $p<0.01$). Given that an R value of 1 indicates a high level of separation, the separation in this data set is moderate, which is seen by the overlapping of the groups polygons in Figure 3.2. The NMDS indicates that Wellington sites may be of better quality as pollution sensitive taxa such as the mayfly *Deleatidium*, were associated with a number of Wellington sites. Those species that are considered pollution tolerant such as the caddisfly *Oxyethira* and the snail *Potamopyrgus* spp., have

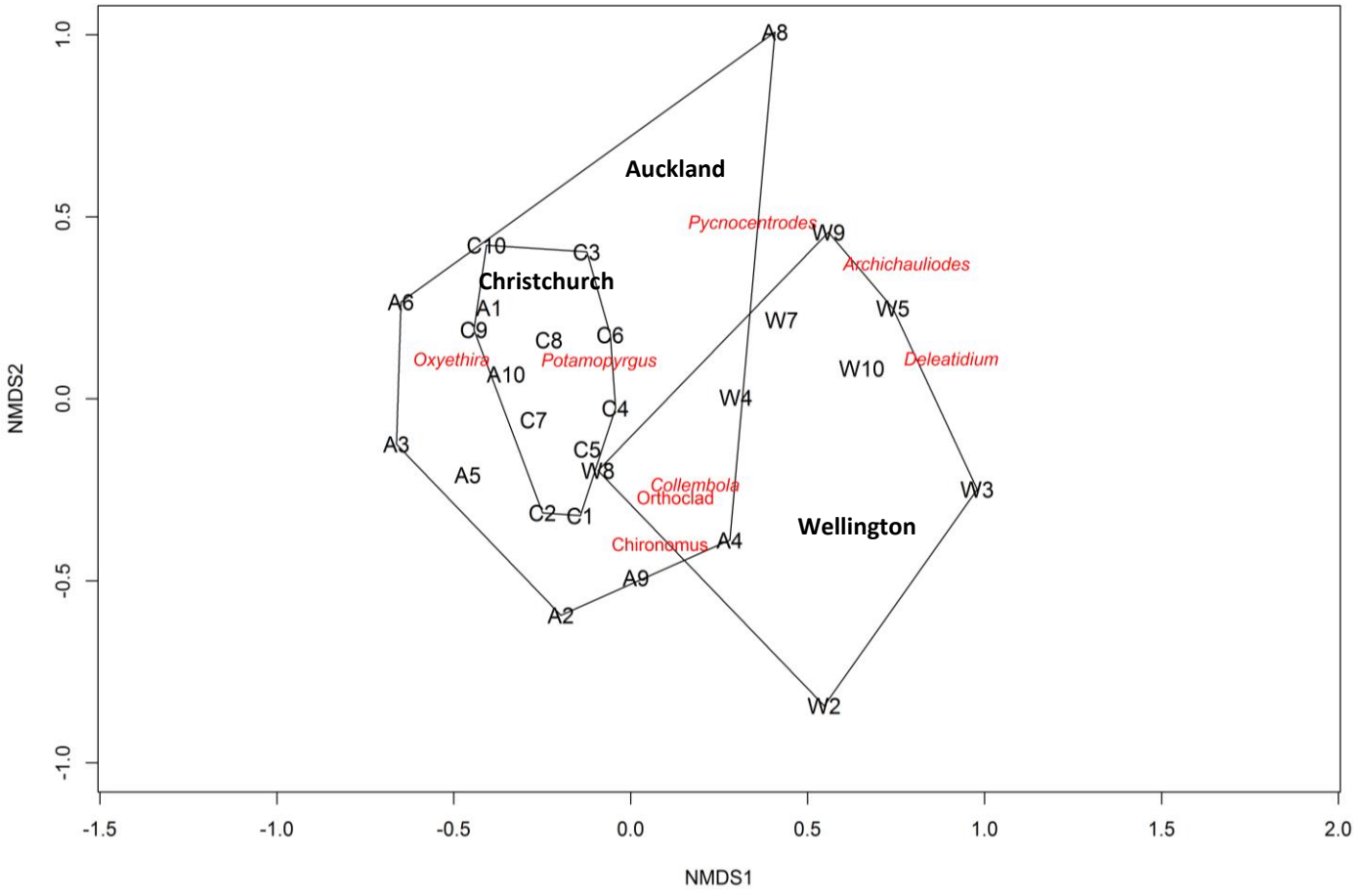

Figure 3.2: NMDS of invertebrate community data with binary Bray-Curtis distance, stress = 0.20. Polygons represent cities Auckland, Christchurch, and Wellington.

lower NMDS1 axis scores associated with the Auckland and Christchurch sites. An Auckland site (A8) was highly separated from all other sites. This may be driven by the absence of crustacea and diptera at this site, whereas at least one of these macroinvertebrates are represented at all other sites.

The envfit function was used to investigate which environmental parameters may influence the benthic macroinvertebrate community (Table 3.3). The sediment metal index (p <0.01), DO (p <0.05) and temperature (p <0.01) influenced the presence or absence of benthic macroinvertebrate taxa. All other parameters were not significant.

Table 3.3: Results from the envfit function on the benthic macroinvertebrate community presence/absence data. Values in bold indicate statistical significance.

Environmental Variable	r^2	Pr(>r)
Sediment metal index	**0.436**	**0.002****
Water metal index	0.060	0.475
pH	0.094	0.299
Conductivity	0.135	0.174
Temperature	**0.427**	**0.004****
DO	**0.311**	**0.012***
SI	0.150	0.158
Depth	0.182	0.097
Width	0.035	0.664
Latitude	0.019	0.808
Impervious surface area	0.012	0.854

Stars denote significance, * p <0.05, ** p <0.01

A CCA was performed on the benthic invertebrate community data with the sediment metal index, temperature and DO as parameters. The environmental parameters are represented by vector arrows which point in the direction of maximum change of that variable across the ordination diagram. As explained in Chapter 2, the sediment metal index values increase as concentrations of combined metals decrease. Therefore, those sites towards the top left corner had lower sediment metal concentrations (Figure 3.3). The bottom right quadrat where sites W8, A2, and C1 are situated, represents high sediment metal concentrations. The CCA suggests that Wellington sites were characterised by higher DO compared to Auckland and Christchurch, driving the DO vector. Conversely, Christchurch and Auckland sites had higher temperatures than Wellington sites. This ordination explains 17.9% of the total variance in the community data.

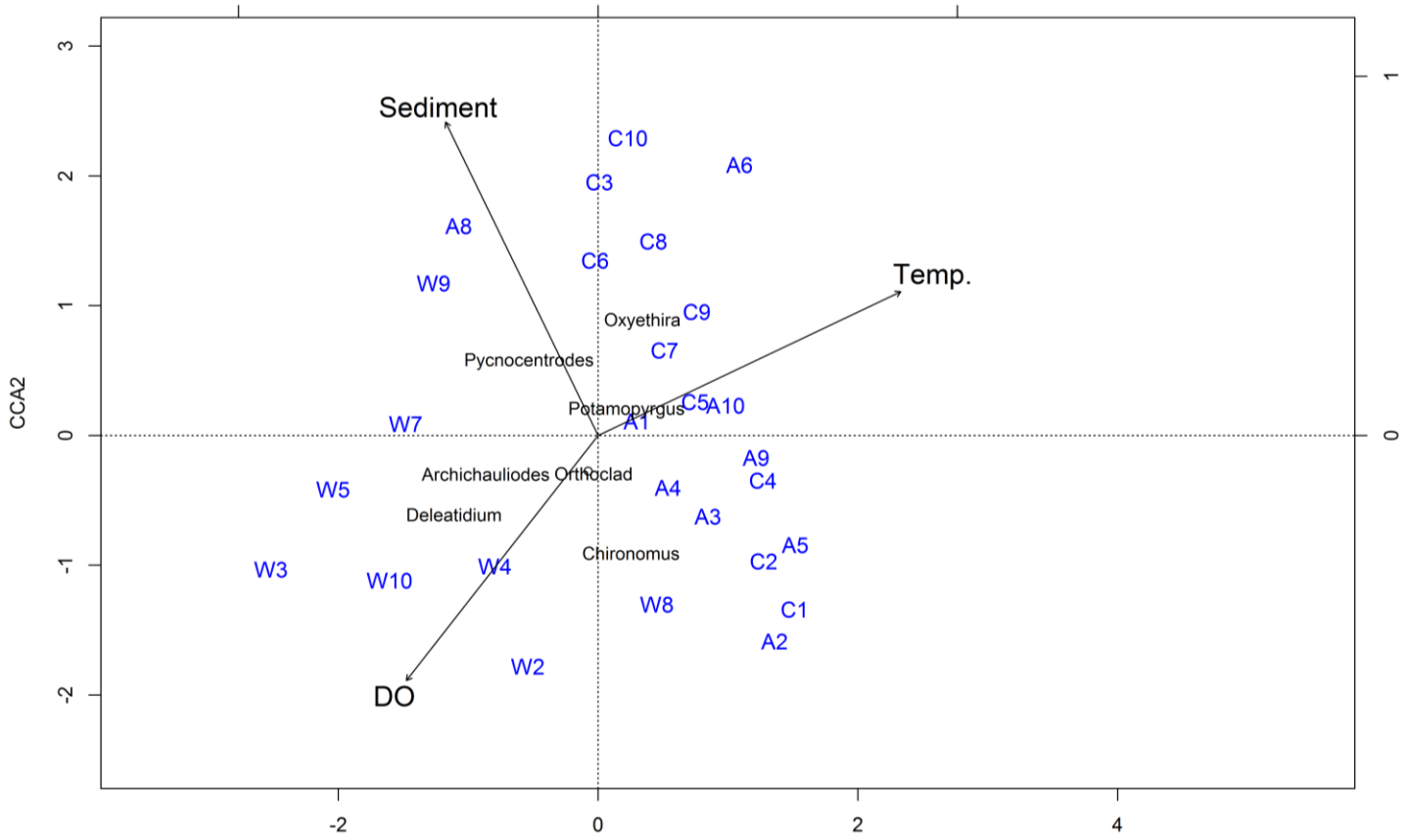

Figure 3.3: CCA of benthic invertebrate community data constrained by the sediment metal index (Sediment), DO and temperature (Temp.) environmental variables. Total variance explained by the constrained ordination is 17.9%.

Comparison of cities

A range of biotic indices were calculated for the 30 sites, these were taxonomic richness, number of EPT taxa, MCI and the UCI. Wellington generally had higher values for all the biotic indices, indicating better stream health (Figure 3.4). Taxonomic richness was significantly lower in Auckland than Wellington and Christchurch sites, with mean values of 7.6, 12.8 and 11.8 respectively (one-way ANOVA, F=6.5, df=2, p <0.01). Auckland also had significantly lower values for the number of EPT taxa compared to Wellington (one-way ANOVA, F=4.6, df=2, p <0.05). Auckland values ranged from zero to five while Wellington had values from zero to eight. Christchurch was not significantly different to either Auckland or Wellington with the number of EPT taxa ranging from zero to six.

The MCI and UCI were both significantly higher in Wellington compared to Auckland and Christchurch (one-way ANOVA, F=8.8, df=2, p <0.01) (one-way ANOVA, F=8.4, df=2, p <0.01). The MCI average for Auckland and Christchurch was below 80, suggesting overall 'poor' water quality in these two cities. In contrast, Wellington had an average over 100, suggesting 'good' water quality overall in the city. The UCI gave a similar result, Auckland and Christchurch had values of almost zero and below zero respectively, while Wellington was well above zero.

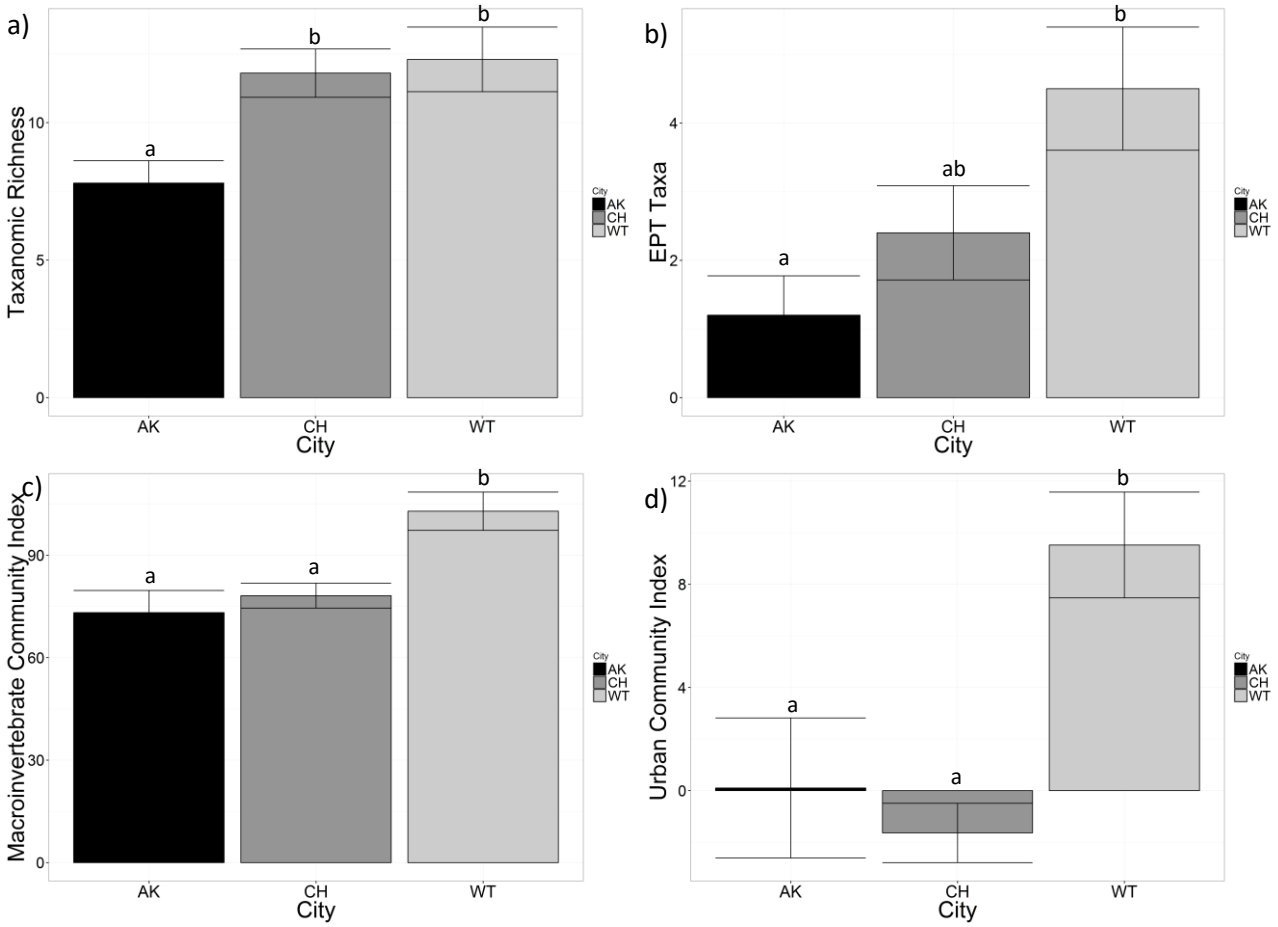

Figure 3.4: Mean values of biotic indices a) taxonomic richness, b) EPT taxa, c) MCI, and d) UCI for Auckland, Christchurch, and Wellington. Letters denote significant differences and error bars are ± 1 S.E.

3.3.4 Relationships between heavy metals and other environmental variables with biotic indices

Stepwise model selection was used to investigate the environmental variables that best describe the benthic invertebrate indices. The sediment metal index was significant for three out of the four biotic indices (EPT taxa, MCI, and UCI) models, with relative contributions in the MCI and UCI at 16.6% and 15.4%. The water metal index was significant only for the UCI and had the lowest contribution out of all predictors. In contrast, taxa richness showed that only the latitude variable had a significant effect indicating that the location of the cities was the reason for changes in taxa richness. The MCI, UCI, and number of EPT taxa all had DO and pH as significant predictors of the benthic invertebrate indices as well as the metal indices (Tables 3.4 and 3.5).

Table 3.4: Stepwise GLM model selection results for taxa richness and EPT taxa.

Response	Predictors	Pr(>|z|)
Taxa Richness	Latitude	<0.01
Null Deviance	**Residual Deviance**	**Pseudo R2**
34.02 on 26 DF	24.24 on 25 DF	0.287
EPT taxa	Sediment index	<0.01
	DO	<0.01
	pH	<0.01
Null Deviance	**Residual Deviance**	**Pseudo R2**
79.95 on 26 DF	25.90 on 23 DF	0.676

Table 3.5: Stepwise multiple linear regression results for MCI and UCI.

Response	Predictors	Pr(>|t|)	Relative importance (%)
MCI	DO	<0.01	60.5
	pH	0.01	22.9
	Sediment index	0.03	16.6
F Statistic		**p value**	**Adjusted R2**
16.86 on 3 and 23 DF		<0.01	0.647
UCI	DO	<0.01	42.2
	pH	<0.01	32.0
	Sediment index	<0.01	15.4
	Water index	<0.01	10.5
F Statistic		**p value**	**Adjusted R2**
25.37 on 4 and 22 DF		<0.01	0.790

3.3.5 Benthic invertebrate communities within each city

A gradient of stream quality was observed within each city (Figures 3.5 – 3.7). Sites are ordered by the sediment metal index determined in Chapter 2, from least contaminated to most contaminated (left to right). Auckland sites appear to be generally degraded with taxonomic richness reaching only 13 and as low as four across the nine sites. The sensitive EPT taxa were absent at four of the sites with only two sites having more than one. The MCI indicates 'poor' water quality for seven out of the nine sites and the UCI had a similar pattern (Figure 3.5).

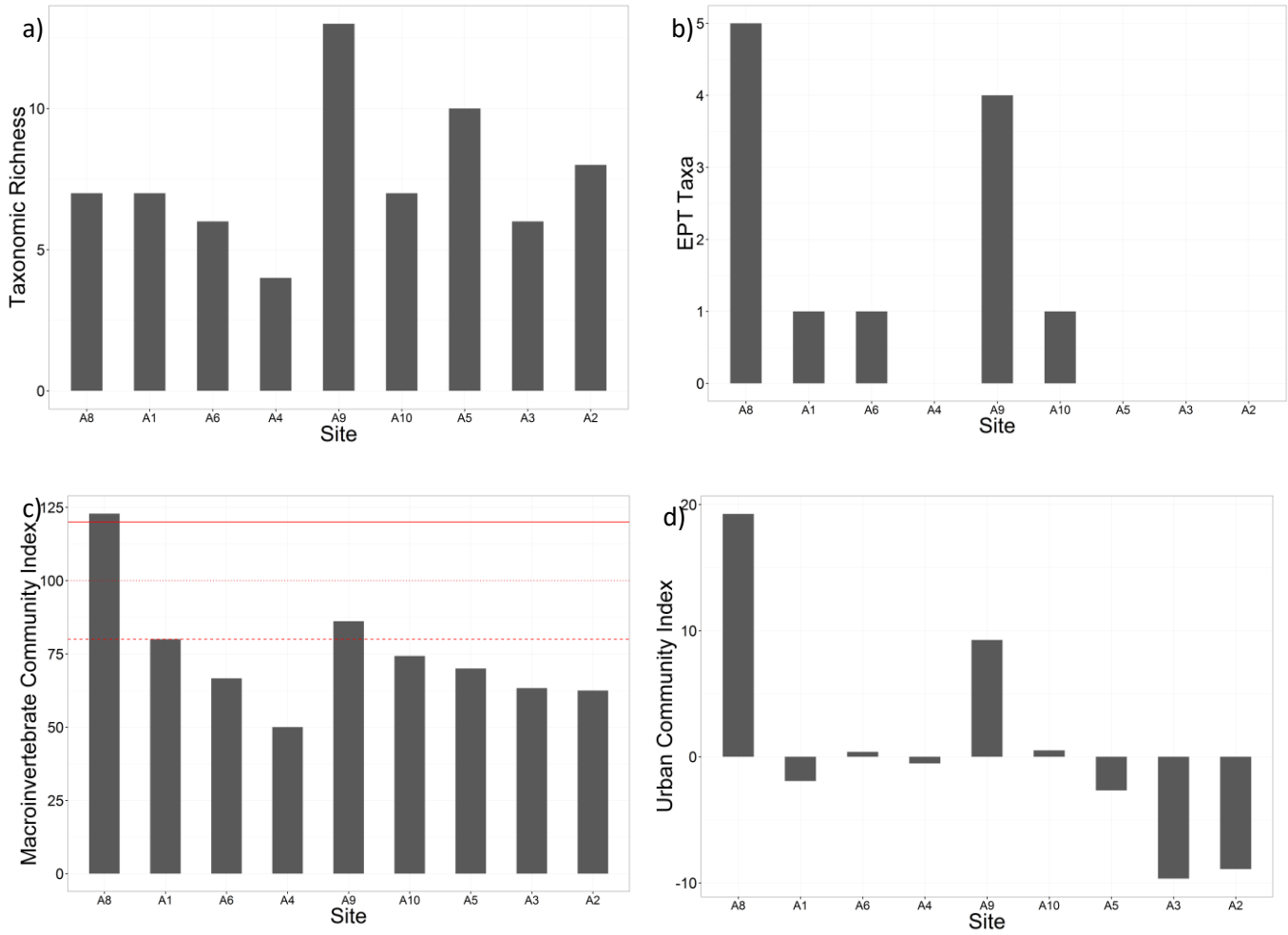

Figure 3.5: Auckland biotic indices for individual sites. a) taxonomic richness, b) EPT taxa, c) MCI - lLines on MCI plot represents the different classes of quality, below dotted = 'poor', dashed line = fair/good threshold, solid line = excellent threshold, d) UCI. Sites are ordered from best to worst (left to right) according to the sediment metal index.

Christchurch sites had similar biotic indices to Auckland. Taxonomic richness was variable across Christchurch ranging from seven to 16 (Figure 3.6). The EPT taxa were absent at three sites, which are not necessarily those with lower taxonomic richness. Although EPT taxa were present at Christchurch sites, there were no Ephemeroptera or Plecoptera orders. The MCI scores showed little variation, with most below 80 i.e. 'poor' water quality. Three sites reached 'fair' water quality on the MCI criteria, these sites are also three out of the four that had positive UCI scores.

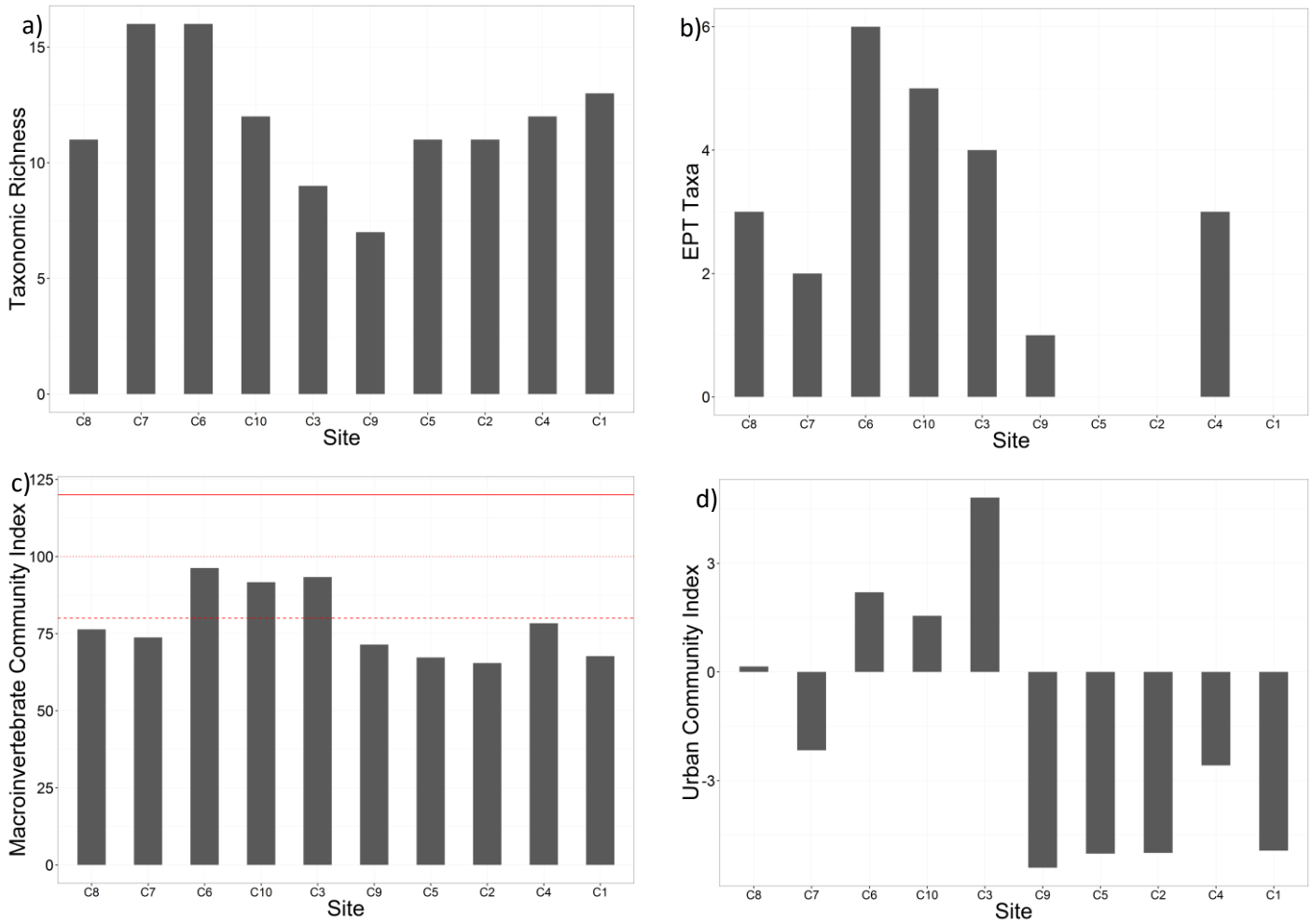

Figure 3.6: Christchurch biotic indices for individual sites. a) taxonomic richness, b) EPT taxa, c) MCI - lLines on MCI plot represents the different classes of quality, below dotted = 'poor', dashed line = fair/good threshold, solid line = excellent threshold, d) UCI. Sites are ordered from best to worst (left to right) according to the sediment metal index.

According to the biotic indices, Wellington sites generally had better stream quality. The sensitive EPT taxa were absent only at one site (Figure 3.7). In contrast to Christchurch, the EPT taxa generally included taxa from at least two of the three orders. The same site that was absent of EPT taxa was also the only one to be of 'poor' quality for both the MCI and UCI. A gradient of stream ecological health in Wellington was observed, however, it was largely centred at better ecological health than Auckland and Christchurch.

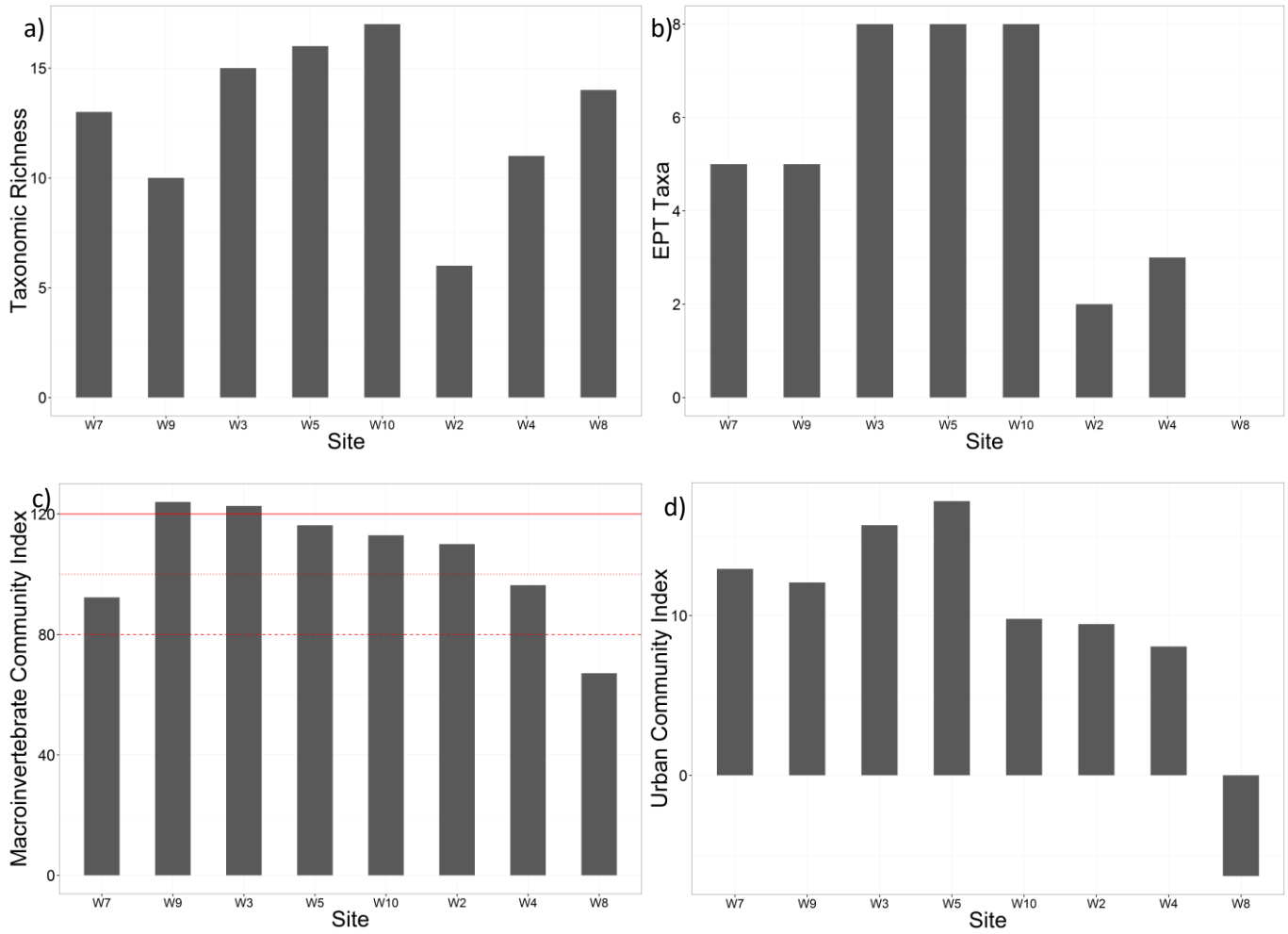

Figure 3.7: Wellington biotic indices for individual sites.. a) taxonomic richness, b) EPT taxa, c) MCI - lLines on MCI plot represents the different classes of quality, below dotted = 'poor', dashed line = fair/good threshold, solid line = excellent threshold, d) UCI. Sites are ordered from best to worst (left to right) by the sediment metal index.

3.3.6 Relationships between heavy metals and other environmental variables with biotic indices within each city

Stepwise model selection was also performed for each city to investigate whether patterns were the same across the three cities as they are within the individual cities. Across the three cities none of the environmental variables explained taxonomic richness (Table 3.6). The MCI, UCI and EPT taxa model selection for each city had variable environmental predictors and no consistent common predictor (Tables 3.6 and 3.7). However, the water and sediment metal indices appeared in all three cities for various indices, EPT taxa for Christchurch and Wellington, and MCI and UCI for Auckland. The r^2 and pseudo-r^2 values were all above 0.5 indicating good fits for all the significant models. The only environmental variables to not be significant were depth, width, velocity and substrate index.

Table 3.6: Stepwise GLM model selection for biotic indices taxa richness and EPT taxa.

	Response	Predictors	Pr(>|z|)
Auckland	**Taxa Richness**	ns	ns
	EPT taxa	pH	<0.01
	Null Deviance	**Residual Deviance**	**Pseudo R2**
	20.28 on 8 DF	9.90 on 7 DF	0.511
Christchurch	**Taxa Richness**	ns	ns
	EPT taxa	DO	<0.01
		Water index	0.02
		Temperature	0.03
	Null Deviance	**Residual Deviance**	**Pseudo R2**
	22.62 on 9 DF	7.23 on 6 DF	0.680
Wellington	**Taxa Richness**	ns	ns
	EPT taxa	Sediment index	<0.01
		Water index	<0.01
		pH	0.01
		Conductivity	<0.01
	Null Deviance	**Residual Deviance**	**Pseudo R2**
	17.80 on 7 DF	2.65 on 3 DF	0.851

Table 3.7: Stepwise multiple linear regression model selection results for biotic indices MCI and UCI.

	Response	Predictors	Pr(>|t|)	Relative importance (%)
Auckland	**MCI**	Sediment index	0.04	NA
	F Statistic		**p value**	**Adjusted R2**
	6.78 on 1 and 7 DF		0.04	0.420
	UCI	Temperature	<0.01	10.0
		Water index	0.03	24.6
		Sediment index	<0.01	25.3
		pH	<0.01	40.0
	F Statistic		**p value**	**Adjusted R2**
	119.4 on 4 and 4 DF		<0.01	0.983
Christchurch	**MCI**	% Impervious	<0.01	NA
	F Statistic		**p value**	**Adjusted R2**
	25.51 on 1 and 8 DF		<0.01	0.732
	UCI	% Impervious	<0.01	NA
	F Statistic		**p value**	**Adjusted R2**
	41.03 on 1 and 8 DF		<0.01	0.816
Wellington	**MCI**	DO	0.01	NA
	F Statistic		**p value**	**Adjusted R2**
	11.48 on 1 and 6 DF		0.01	0.600
	UCI	ns	ns	ns

3.4 Discussion

The benthic invertebrate communities found in this study were comparable to previous descriptions of urban streams, where diversity is reduced as is the number of sensitive or EPT taxa (Morse *et al.* 2003; Allan 2004; Meyer *et al.* 2005). In this study the highest number of taxa at an individual site was 17. The number of EPT taxa reached eight at three sites in Wellington, however, all other sites were six or less. Taxonomic richness in New Zealand streams from native forest or pastoral catchments, have a large range of values. In this study the taxonomic richness was comparable to the lower range of values in previous survey studies, but much lower than the upper reported values of 20 to 56 taxa and averages (Quinn and Hickey 1990; Harding and Winterbourn 1995; Collier *et al.* 1998; Collier and Quinn 2003). Morse *et al*. (2003) found that streams draining catchments > 6% total impervious area did not have total richness exceeding 18 taxa or the number of EPT taxa to exceed six which largely agrees with the findings of this study.

The most common taxa found were primarily snails and worms, *Potamopyrgus* spp. and Oligochaeta. No taxa from the orders of Ephemeroptera, Plecoptera, or Trichoptera were found. Snails and worms

are known to be pollution tolerant benthic invertebrates, as indicated by their MCI score (Stark 1985). The common occurrence of these taxa across all the sites is reflective of the poor quality of urban streams. Results from various New Zealand surveys in streams with minimal human impact, often report the mayfly *Deleatidium* spp. to be the most common as well as genera of the trichopterans (Quinn and Hickey 1990; Death and Joy 2004). The common occurrence of the *Potamopyrgus* spp. and Oligochaeta confirms observations from other studies in urban streams (Hall *et al.* 2001; Suren and McMurtrie 2005; Paul and Meyer 2008), demonstrating the consistent effects of urbanisation on the benthic invertebrate community. The most diverse order of benthic invertebrates was Trichoptera. In Christchurch, this was most commonly *Hudsonema* spp. and *Triplectides* sp., whereas the net-spinning caddisflies were most commonly found in Wellington. This agrees with a number of other studies across different land uses where Trichoptera and Diptera are often the most diverse (Quinn and Hickey 1990; Collier *et al.* 1998; Suren and McMurtrie 2005). The use of EPT taxa as an indication of pollution has been argued by researchers, as often, the more sensitive Ephemeroptera are replaced by pollution tolerant Trichoptera taxa (Clements and Kiffney 1994). Therefore, suggesting that the EPT metric can be skewed by the presence of more pollution tolerant Trichoptera taxa.

Published large-scale urban stream studies in New Zealand are relatively few, as are studies comparing multiple cities. In particular, no studies comparing multiple cities could be found in New Zealand. However, several city and regional councils have monitoring programmes for a selection of urban streams in their region. The level of identification differed between the Auckland council data and that of this study, making comparisons of taxonomic richness and the number of EPT taxa difficult. However, MCI scores are comparable with many sites in the urban area being classed as 'poor' (Auckland Council, 2015). Taxonomic richness in Christchurch was similar in this study compared to data from the Christchurch City Council, which ranged between five and 17 (Blakely 2014). The number of EPT taxa was also generally higher, resulting in higher MCI scores. However, given that the majority of sites were classed as 'poor' and few as 'fair', the higher scores are not particularly evident of better stream health. The EPT taxa were entirely made up of taxa from Trichoptera, this is consistent with the Christchurch City Council reports (McMurtrie 2009; Blakely 2014) and also reported in other studies in Christchurch (Suren and McMurtrie 2005). Overall, Wellington sites in this study had better stream health based on the biotic indices, with more diverse EPT taxa and higher MCI scores. Conversely, in the latest monitoring report from the Greater Wellington Regional Council has six of the seven urban streams were classed in the 'poor' or 'fair' MCI category (Heath *et al.* 2014).

While there were no significant differences for biotic indices between Auckland and Christchurch, the NMDS ordination indicates that the community composition for Christchurch was a subset of the Auckland benthic invertebrate commuinties. Auckland communities appear to be highly variable

between sites when compared to Christchurch. This may be due to geographical differences, however, the latitude predictor variable was not significant in the model selection. This does not rule out the influence of a 'city effect'. The limitations of the stepwise multiple regression are well discussed within statistical literature, the order of parameter entry and the number of parameters can affect the selected model (Whittingham *et al.* 2006). This is identified as a particular problem if the parameters are correlated. If there is in fact a 'city effect', it may be masked by the inclusion of DO or other parameters.

The differences between cities is further exemplified by the Wellington data, where the NMDS ordination and biotic indices show significant differences. Sampling in Wellington occurred approximately one week after a significant flooding event and this may have caused changes in community composition. However, it was also observed that the CBD of Wellington and surrounding residential areas appeared to be more compact and had more forested and permeable areas than Auckland and Christchurch. This may mean that urban effects on streams were concentrated to a smaller area. While no literature could be found for comparison, the impervious area data extracted from the FENZ dataset had seven out of 10 sites below 50% impervious area for Wellington (compared to two and five sites <50% impervious area for Christchurch and Auckland respectively). The three sites above 50% were also determined to be the most polluted by the PCA sediment metal index and had the lowest MCI, UCI and EPT taxa scores in Wellington, for example, at the Woburn Road (W8) site, which was the most contaminated site out of all three cities based on the PCA ordination.

3.4.1 Invertebrate and metal relationship discussion

The results from this study suggest that sediment metals have a significant effect on the benthic invertebrate communities in urban streams. The sediment metal index was identified in the CCA to be significant. However, the metal indices that were identified in the model selection for biotic indices, contributed little to the overall models. Taxonomic and Ephemeroptera richness is often reported as a good indicator of heavy metal contamination (Hickey and Clements 1998; Beltman *et al.* 1999; Beasley and Kneale 2003; Allan 2004). In this study, no significant models were found for taxa richness in the individual cities, and only latitude was identified for model selection with all three cities combined. When comparing the PCA metal indices with the presence of mayflies, no significant patterns were observed. The diversity of Ephemeroptera in this study was sparse, with only five sites out of the 30 having more than one genus and 22 of the sites had no Ephemeroptera present. Therefore, it is not surprising that when investigating the effectiveness of Ephemeroptera richness as an indicator of metal contamination for this dataset, no significant patterns were observed.

The lack of relationship between taxa richness and heavy metal contamination as well as the lack of Ephemeroptera diversity may be due to the fact that the sites in this study were markedly degraded

and exceeded the threshold of impervious cover that effects the benthic invertebrate community (Morse *et al.* 2003; Walsh *et al.* 2005; Paul and Meyer 2008). All sites had impervious cover greater than 10%, which is close to or above thresholds that significantly change benthic invertebrate communities reported in literature (Morse *et al.* 2003; Allan 2004; Paul and Meyer 2008). It may be that these sites are heavily impacted by other factors associated with urbanisation such as hydrological changes, that mean the more sensitive taxa such as the Ephemeroptera, will be absent whether there are high concentrations of metals or not. Hickey *et al.* (1998) found responses of New Zealand benthic invertebrates to metals to be similar to those overseas. They found that the number of mayflies, species richness of mayflies, number of EPT taxa, and total taxonomic richness were the most important variables for separation of reference and metal-polluted sites. However, the sites used in the Hickey *et al.* study were in the Coromandel region, where there was limited urbanisation. This makes the effects of heavy metals on benthic invertebrates more easily identifiable compared to this study, where there are a number of pressures associated with urban streams that may have overriden the effects of metals.

In an ideal study design, all in-stream habitat variables would have been kept constant, however it was not possible to find sites that fitted this criterion. A gradient of heavy metal concentrations from low to high was sought within each city. As a result, some of the sites chosen had high amounts of fine sediment or artificial banks as these were in areas that were expected to have high metal concentrations. Considering that many urban streams have been managed and treated as drains, it is not surprising that a number of potential and chosen sites had these qualities. Therefore, general habitat variables were also recorded and measured to later account for these variations. Dissolved oxygen was the most consistent predictor in the model selections for the biotic indices, contributing the most to the models, and was also identified as a significant variable in the ordinations. However, when investigating the individual cities, DO is only significant for two models out of the 12. This suggests that there was a 'city effect' as suggested above, yet when the latitude term is included in the full dataset it is not significant and DO is still identified as a significant predictor. It may be that the inclusion of all three cities provides a large enough range for both DO and the biotic indices for effects to be seen. It must also be considered that the DO metric is based on a spot measurement and varies during the day. Therefore, the significance of DO in this data should be interpreted with care.

The water metal index was identified as a significant predictor for UCI and EPT as well as UCI scores in Auckland alone and EPT taxa in Christchurch and Wellington. However, the CCU was not found to be a significant predictor for any of the biotic indices. This is in contrast to the findings of Clements *et al.* (2000) where water heavy metal concentration was an important predictor for nearly all the benthic invertebrate community variables. Changes in invertebrate communities were seen at medium and

high metal contamination, as defined by the CCU, and no changes were observed at low contamination. This may explain the discrepancy between results, as only two sites reach just above the low contamination CCU category in this study, with many sites below one.

According to both the pollution load index (PLI) and risk index (RI), few sites showed metal contamination above background values in the sediment, although many exceeded guidelines for various metals (discussed in Chapter 2). There were also no significant relationships between the two indices and the biotic indices. However, the sediment metal index from the PCA was significant in explaining the benthic invertebrate communities (CCA) and was a significant predictor for EPT taxa, MCI, and UCI for the full dataset, as well as the only predictor for Auckland MCI and a further two models. As discussed in Chapter 2, the maximum background concentrations were used for the PLI and RI. The range for some of the heavy metals were reasonably large and therefore, may be more polluted than calculated here. This could explain the significance of the PCA sediment metal index compared to the standardised metal indices, PLI and RI.

The MCI and UCI models are multiple linear regressions, therefore, the relative contribution of each predictor can be calculated. Aside from the Auckland MCI, where the sediment metal index is the only predictor, the contribution of the sediment index is less than 20% for UCI and MCI in the full dataset and less than 25% for Auckland UCI. This suggests that while other environmental variables (DO and pH in this case) have a stronger effect on the biotic indices, there is still some variation explained by metal pollution.

The water metal index was identified as a significant predictor in four models and the sediment metal index for six models, of these, three had both the water and sediment index. The relative contributions for two of these had the sediment metal index contributing more than the water metal index. This, in conjunction with the sediment metal index being significant in the CCA, could indicate that sediment metal concentrations may be a better monitoring tool than water concentrations. Concentrations of heavy metals in the sediment of receiving waters accumulate and can reach concentrations orders of magnitude greater than present in the overlying water (Maltby *et al.* 1995), providing a measure of metal pollution over time. However, it must also be considered that the amount of variation explained in the water metal index (i.e. PC2) is less than for the sediment index (i.e. PC1). Therefore, the water metal index may not explain water metal contamination as well as the sediment metal index does for sediment metal contamination.

Interestingly, Christchurch's MCI and UCI were the only metrics to identify impervious surface area as a significant variable. Substantial literature identifies a significant relationship between benthic invertebrate communities and impervious surface area, where diversity and sensitive taxa decrease

as impervious cover increases (Klein 1979; Pratt *et al.* 1981; Thorne *et al.* 2000; Morse *et al.* 2003; Paul and Meyer 2008). The impervious surface area for each site in this study were all above 10%, and as mentioned above, this is higher or close to, the thresholds found in much of the literature. Therefore, this may account for the lack of relationship between the two.

3.5 Conclusions

The benthic invertebrate communities found in this study were similar to those found in literature, where diversity is decreased and pollution tolerant taxa are common. There were significant differences found between the cities. This was largely for Wellington, where biotic indices were often better and the overall communities in Wellington were significantly different as identified by the NMDS. While it would seem likely that this difference between cities could be attributed to the geography of the cities, the statistics do not identify latitude as a significant predictor. However, it was noted that the Wellington urban area appeared to be more 'compact'.

Although taxa richness is often reported to be a useful indicator of metal pollution, it was not in this study. However, sediment metals appeared to be a significant predictor of invertebrate communities. The contribution of sediment metals to explaining the variation in invertebrate communities was relatively small, at around 20%, yet it was statistically significant. The common pollution indices (CCU, PLI, and RI) did not show any significant relationships with the invertebrate communities. None of the biotic indices were strong indicators for metal pollution, which is likely due to the multiple stressors in urban environments clouding the relationships.

4 Chapter 4: Bioaccumulation of Cu and Zn by the *Deleatidium* spp. mayfly.

4.1 Introduction

Freshwater invertebrates are commonly used as indicators of the effects of metals on stream biota as they include taxa that can be highly sensitive to metal toxicity and provide good indicators of the overall health of the stream biota. They can be assessed both through field surveys of contaminated systems or laboratory ecotoxicological studies. Chapters 2 and 3 focussed on a field survey, while this chapter will describe a laboratory study on the impacts of specific metals (Cu and Zn) to a specific benthic invertebrate (*Deleatidium* spp.). Determining the effects of metals on freshwater invertebrates can be challenging. There are often discrepancies between results from field and laboratory studies on the toxicity of heavy metals on benthic invertebrates in freshwater systems (Brix *et al.* 2011). Field studies in metal contaminated streams show significantly reduced benthic invertebrate diversity and abundance (Hickey and Clements 1998; Harding 2005; Pollard and Yuan 2006), while laboratory studies often indicate that many benthic invertebrates may be relatively insensitive towards some heavy metals, except at high concentrations (Clements *et al.* 2013). There are three pathways by which organisms can take up trace metals. These pathways are respiration across gill or skin surfaces, adsorption onto the body surface, and the ingestion of food in which trace metals have already accumulated (Beltman *et al.* 1999). Typically, laboratory tests are conducted using water exposures rather than diet. This difference in exposure pathway may partially explain the discrepancy between laboratory and field results. Diet exposure may be more toxic than dissolved metals in the water (Luoma and Rainbow 2005; Martin *et al.* 2007). Therefore, multiple approaches are needed to tease apart impacts.

Over the last decade, there have been a number of freshwater studies investigating the accumulation and toxicity of metals through diet exposure in different benthic organisms (Cid *et al.* 2010; Cain *et al.* 2011; Xie and Buchwalter 2011). The majority of these studies have focussed on Cd accumulation (Xie *et al.* 2010) and have been performed on species that do not occur in New Zealand. Heavy metal accumulation in benthic invertebrates can be highly variable (Dallinger and Rainbow 1993). Benthic invertebrates living in the same habitat can have very different body tissue concentrations of trace metals (Rainbow 2002). The concentrations of metals can vary with the species, organism size, life stage and the source of the organism as well as the metal species (Rainbow 2002; Luoma and Rainbow 2005). Therefore, if we are to understand the effects of metals on New Zealand stream communities, studies on native and endemic invertebrate species are important.

The common New Zealand leptophlebiid mayfly, *Deleatidium* spp., is considered a pollution sensitive endemic taxa found in abundance across New Zealand and particularly the South Island (Harding *et al.* 1997). *Deleatidium* spp. are grazers that feed on diatom algae and other organic matter from the surface of cobbles (Winterbourn *et al.* 1984), making them an ideal species for monitoring accumulation through diet. Although it has not been widely used as an ecotoxicological test organism, it has proved useful in other New Zealand studies (Hickey and Vickers 1992). It's abundance in nearby streams and rivers close to Christchurch allow large numbers to be collected as required for use in replicated experiments.

Stream substrate is typically covered by a layer of biofilm composed of bacteria, algae, fungi, protozoa and other microscopic organisms in a complex polymer linked assemblage (Farag *et al.* 2007). Biofilm is ubiquitous in freshwater systems and is a major source of energy and food for stream food webs (Meylan *et al.* 2004). These biofilms are natural sinks for heavy metals and have been frequently found to be enriched with copper and zinc in urban streams compared to sediments (Ancion *et al.* 2013). Standardized diets, such as single algal species, are often used in ecotoxicological laboratory tests to reduce experimental variability. However, the use of natural biofilms more closely reflects the complex diets that many organisms experience in nature (Meylan *et al.* 2004).

This study compared the uptake of two metals (Cu and Zn), through exposure dissolved in stream water and through consumption of contaminated biofilm. Concentrations used in these experiments were selected based on literature, guidelines (ANZECC 2000), and survey data (Chapter 2). The ANZECC 95% protection guideline was used in both the Cu and Zn experiments. The upper concentrations found in the survey for Cu and Zn were at 3.9 $\mu g\ L^{-1}$ and 50 – 150 $\mu g\ L^{1}$ respectively. For Cu, the highest concentrations were selected based on LC50 data for various benthic invertebrates in literature, these were reported to be up to 300 $\mu g\ L^{-1}$ (Milani *et al.* 2003; Brinkman and Johnston 2008). The LC50's for Zn varied from around 1000 – 5000 $\mu g\ L^{-1}$ (Balch *et al.* 2000; Brinkman and Johnston 2008; Mebane *et al.* 2012), determining the concentrations 1500 and 5000 $\mu g\ L^{-1}$.

Heavy metals are often essential ions but once the concentration exceeds normal concentrations, they have the potential to become toxic (Luoma 1983). Both Zn and Cu are essential ions. Zinc has been described as a co-factor in many enzyme reactions, similarly copper occurs, for example, in hemocyanin (present in crustaceans and molluscs) (Dallinger and Rainbow 1993). Biofilms are intensely grazed by benthic invertebrates and have been suggested to provide an important pathway for Cu and Zn into the food chain (Farag *et al.* 2007; Ancion *et al.* 2013).

There is no standardised method for conducting diet metal exposures, with many studies growing single-cell, algal species for the experiment. However, an increased emphasis on understanding the

bioavailability of biologically incorporated metals and has resulted in an increase in studies that have evaluated metal-diet bioaccumulation via a natural diet (DeForest and Meyer 2015). Few studies have compared simultaneous waterborne and dietborne metal exposures and even fewer where the test organism is exposed to the same waterborne-metal concentration to which its food was exposed (DeForest and Meyer 2015).

The aims of this study were to:

- Compare bioaccumulation of Cu and Zn between diet and dissolved water exposures for the mayfly, *Deleatidium* spp.
- Compare bioaccumulation from single metal exposure (Cu and Zn) and a metal mixture of two metals (Cu and Zn) by the *Deleatidium* spp. mayfly.

4.2 Methods

This study composed of three experiments testing the accumulation in a mayfly for, a) Cu from spiked water and biofilm, b) Zn from spiked water and biofilm, and c) a combined Cu and Zn mixture from water and biofilm. The experiments were conducted on the common New Zealand stream mayfly, *Deleatidium* spp.. Mayfly nymphs were collected from Cust Main Drain (172 37.609 E, 4322.327 S) where no known metal contamination has been recorded. The average water concentration of Zn and Cu at the site (Cust Main Drain) was 3.0 and 0.46 µg L^{-1} respectively (n=3). Water hardness was 0.60 mmol L^{-1} and pH 7.5.

The mayflies were collected using a kicknet and transported in the source stream water with cobble substrate in aerated buckets and returned to the laboratory. Approximately 20 kicks were taken across the stream to ensure at least 340 individuals were collected. Organisms were collected the morning of the beginning of experiment. Nymphs of a similar size were selected and those with dark wing pads were excluded to reduce the possibility of nymphs emerging as adults during the experiments.

Organisms were exposed to contaminated water and biofilm separately at six different concentrations for Cu, Zn, and mixtures of Cu and Zn. Each experiment was run for 96 hours in a temperature control room at 15°C and on a 12:12 hour (light:dark) cycle. Water and mayfly samples were taken at the beginning of the experiment and at 96 hours to be analysed for metals using ICP-MS

4.2.1 Water metal exposure

The accumulation of metals Cu and Zn in mayflies through uptake from water was investigated. A total of six treatment concentrations were used for each experiment. The concentrations were as follows; for the Cu experiment, mayflies were exposed to nominal dissolved concentrations of 0, 1.4, 5, 80,

300 and 1000 µg/L. Initial concentrations of dissolved Cu exposures were within 25% of the nominal concentrations (Table 4.2). For dissolved Zn, concentrations were 0, 8, 50, 150, 1500, and 5000 µg L^{-1}. Generally, all initial concentrations of exposures were within 25% of nominal concentrations. Concentrations for the mixture exposure (Cu and Zn) were determined based on the results of the separate Cu and Zn exposures. The Zn concentration was held at a constant concentration of 1500 µg L^{-1}, while Cu concentrations were altered using the same concentrations as for the Cu only exposures up to 300 µg L^{-1}. Two controls were used, one with only river water and the other with Zn at 1500 µg L^{-1} and no added Cu. The remaining nominal concentrations were, 1.4 and 1500, 5 and 1500, 80 and 1500, and 300 and 1500 µg L^{-1} of Cu and Zn respectively. Initial water exposure concentrations were generally within 10% of the nominal concentration. Desired metal concentrations were achieved by spiking with stock solutions (1000 or 1 mg L^{-1} $CuSO_4$ and 1000 mg L^{-1} $ZnSO_4$) to 6 L of Cust Main Drain river water. The pH was measured after spiking and values were within an acceptable range of 6.6 – 7.3.

For each treatment there were seven replicate new polycarbonate containers containing 380 mL of spiked river water with four mayflies. At the end of 96 hours, mortality was recorded, and mayflies were collected and rinsed with Milli-Q water before freezing in 5.4 mL vials for analysis. Water samples were collected from the initial spiked solution and at 96 hours (n=3). Samples were filtered through a 0.45 µm membrane filter (Millex (33 mm) sterile filter unit) and acidified with ultra-pure concentrated nitric acid (HNO_3) before ICP-MS analysis.

Nominal and measured concentrations for each treatment are shown in Table 4.2.

4.2.2 Biofilm metal exposure

The biofilm exposure consisted of two consecutive parts, firstly the contamination of the biofilm on the stones, and secondly the mayfly exposure to the contaminated stones.

1) Biofilm contamination

Approximately 200 cobbles of similar size and biofilm coverage were collected from Cust Main Drain, ensuring that any invertebrates were removed. All of the stones were placed in a container lined with plastic and sufficient river water to keep cobbles wet until contamination.

The biofilm was dosed with copper, zinc and a mixture of copper and zinc using the same concentrations as for the water exposures. For each treatment, 30 cobbles selected at random were placed in an acid-washed plastic tray and covered with spiked river water and left to equilibrate for 96 hours.

Quantification of biofilm and metals on cobbles

Three cobbles were set aside and frozen until analysis of the amount of biofilm on each cobble was determined. This method was adopted from Steinman *et al.* (1996). There was not a substantial amount of biomass that could be easily removed, thus vigorous brushing with a hard-bristled toothbrush was used and a slurry with distilled water was created. The slurry was filtered onto a pre-weighed and pre-ashed glass fiber filter. The filters were put in weigh boats and placed inside a drying oven at 105°C for 24 hours. Filters were removed from the oven in their weigh boats and weighed to the nearest 0.1 mg, oxidized at 500°C, and reweighed. The ash-free-dry-mass (AFDM) was calculated using equation 4.1:

$$\mathrm{AFDM} = \frac{\mathrm{W_{a+f}} - \mathrm{W_f}}{\mathrm{SA}} \qquad \textit{Equation 4.1}$$

where $W_{a=f}$ is the mass of the ashed material with the filter, W_f is the mass of the filter, and SA is the surface area of the stone.

The surface area of the stones was determined by tracing the stone onto a piece of paper, weighing the paper and relating to a weighed 1 x 1 cm piece of paper to obtain an approximate cm^2 area (Bergey and Getty 2006).

A dilute-HCl extraction method targeting the labile metal concentration adapted from Snape *et al.* (2004) was used to determine the biofilm concentrations. Randomly selected stones from each concentration, control and before the contamination (n=3), were placed in a new 250 mL polystyrene container and covered with 130 mL of 1M HCl solution. The samples were placed on a shaker table for 4 hours. At the end of the extraction, 10 mL of the extraction solution was filtered through a 0.45 µm membrane filter (Millex (33 mm) sterile filter unit). Samples were refrigerated until analysis for metals by ICP-MS. A blank was included in each batch extraction (n=4).

Results were used in conjunction with the AFDM and surface area of the stones to calculate the µg of metal per mg of biofilm.

2) Mayfly exposure

The contaminated biofilm on stones were transferred into clean water from Cust Main Drain. The set-up for the biofilm exposure was the same as for the water exposure, with seven x 380 ml replicates and four mayflies in each container. Two contaminated stones were placed in each container as the food source and source of exposure for the mayflies. Analysis and sample collection were the same as for the water exposure, with water and mayfly samples collected at the beginning and at 96 hours of the experiment and mayflies rinsed with Milli-Q and placed in 5.4 mL vials for freezing.

4.2.3 Mayfly tissue and water analysis by ICP-MS

Whole body Zn and Cu concentrations were quantified using ICP-MS. Each sample (n=4 mayflies) was dried at 30°C until constant weight was achieved. Dried tissue was then stored at room temperature until further analysis. The tissue was digested by adding 200 µL and 50 µL ultrapure concentrated HNO_3 and HCl acids respectively into the capped 5 mL plastic vials and leaving for 24 hours to pre-digest before refluxing at 80°C for 1 h. The digested samples were made up to a final volume of 5.4 mL using Milli-Q water. Samples were analysed by ICP-MS. QA/QC was achieved by using procedural blanks and a certified reference material (Bovine Liver SRM 1577c). Recoveries for Cu and Zn from the CRM were acceptable, ranging from 99 – 111% recoveries. Filtered and acidified water samples taken from the exposures were analysed by ICP-MS (Agilent 7500cx) as described in Chapter 2. Recoveries for the ICP-MS CRM were within 12% (Table 4.1).

4.2.4 Statistics

Two-way ANOVA analysis was used to determine differences between biofilm metal concentrations and for the metal accumulation of the mayflies. A post-hoc TukeyHSD was used to identify where significant differences were. Significance level was p <0.05 and all data was log transformed to achieve normality and homogeneity of variance in the model residuals.

4.2.5 Quality Assurance/Quality Control

Detection limits and CRM recoveries are presented in Table 4.1 for the water samples, biofilm and mayfly digestions separately.

Table 4.1: QA/QC data for detection limits, certified reference material recoveries for water samples, biofilm and mayfly digestions.

	Water		**Biofilm**		**Mayfly**	
	Cu	**Zn**	**Cu**	**Zn**	**Cu**	**Zn**
Detection limits	0.1 µg L^{-1}	1 µg L^{-1}	0.1 mg kg^{-1}	10 mg kg^{-1}	0.1 mg kg^{-1}	10 mg kg^{-1}
IV SRM 1643 % Recovery (n=3)	99.9	110.6	94.6	88.4	104.3	94.0
Blanks (µg L^{-1})	<0.1	2.7	0.2	<10	0.2	17.5
CRM[1] % recovery (n=6)	**-**	-	-	-	101.9	109.1

[1]Bovine Liver SRM 1577c

4.3 Results

4.3.1 Dissolved metal exposure concentrations

Generally, measured exposure concentrations were within 25% of nominal values (Table 4.2) and test concentrations were consistent within experiments. The lower concentrations often differed more, however, they did not reach values as high as the next nominal concentration

Table 4.2: Nominal and measured mean ± 95% confidence intervals concentrations of water exposure Zn-only, Cu-only and Cu and Zn mixture experiments (n=3).

	Nominal Cu (µg L^{-1})	Measured Cu (µg L^{-1})		Nominal Zn (µg L^{-1})	Measured Zn (µg L^{-1})
Cu only	0	0.6 ± 0.1	**Zn Only**	0	2.4 ± 1.1
	1.4	2.2 ± 1.2		8	15.0 ± 1.2
	5	4.3 ± 0.5		50	57.5 ± 7.4
	80	64.6 ± 2.9		150	175 ± 15.1
	300	258 ± 5.8		1500	1724 ± 153
	1000	849 ± 103		5000	5979 ± 350
Cu & Zn	0	0.9 ± 0.1		0	8.9 ± 2.1
	0	0.9 ± 0.4		1500	1218 ± 62
	1.4	2.2 ± 0.1		1500	1253 ± 107
	5	5.8 ± 0.1		1500	1218 ± 59
	80	97.3 ± 0.4		1500	1224 ± 51
	300	360 ± 14		1500	1201 ± 23

4.3.2 Biofilm uptake of metals

Copper

Initial Cu concentrations in the biofilm were 367 µg g^{-1} DW, and reached 12, 828 µg g^{-1} DW at the highest treatment. There was a significant difference in Cu accumulation of the biofilm between treatments (one-way ANOVA, $F_{6,29}$=36.59, p <0.01). The Cu concentration of the biofilm significantly increased at the 80 µg L^{-1} treatment (TukeyHSD, p <0.01). This treatment had a 3.4-fold increase relative to the 'initial' Cu concentration in the biofilm. The 300 and 1000 µg L^{-1} loadings had a 12- and almost 35-fold increase respectively (Table 4.3).

Table 4.3: Mean ± 95% confidence intervals for Cu biofilm concentration in Cu-only experiment (n=3).

Biofilm Cu concentrations (µg Cu g^{-1} biofilm DW)	
Initial	367 ± 292
Control	292 ± 84
1.4	330 ± 231
5	374 ± 322
80	1239 ± 293
300	4413 ± 555
1000	12828 ± 2292

Zinc

Initial biofilm Zn concentrations were 4661 mg kg^{-1} and reached an average of 83878 mg kg^{-1} at the highest treatment. Biofilm Zn concentrations had greater variation within the treatments. However, there was a significant difference in concentrations between Zn treatments (one-way ANOVA, $F_{5,18}$=72.3, p <0.01). The Zn concentrations of the biofilm significantly increased at the 1500 and 5000 µg L^{-1} treatments, where there was a 5.7- and 6.8-fold increase respectively in Zn concentration (TukeyHSD, p <0.01) (Table 4.4).

Table 4.4: Mean ± 95% confidence intervals for Zn biofilm concentration in Zn-only experiment (n=3).

	Biofilm Zn concentration (µg Zn g^{-1} biofilm DW)
Initial	4661 ± 1177
Control	5176 ± 1490
8	5870 ± 2114
50	4026 ± 1703
150	6653 ± 1230
1500	26471 ± 7433
5000	83878 ± 24430

Copper and Zinc mixture

The Cu concentrations in the Cu and Zn mixture experiment are used for identifying the treatments, as Zn was kept constant. Concentrations of Cu and Zn in the biofilm were comparable to the single metal experiments, ranging from 256 to 3, 397 µg g^{-1} DW for copper and 4, 172 to 31, 823 µg g^{-1} DW for Zn. As for the Cu-only experiment, loading of Cu in the biofilm significantly increased at the 80 µg L^{-1} treatment (TukeyHSD, p <0.01) (Table 4.5). There were no significant differences in biofilm Zn concentrations between all Zn loadings of 1500 µg L^{-1}.

Table 4.5: Mean ± 95% confidence intervals for Cu and Zn biofilm concentration from Cu and Zn mixture experiment (n=3).

	Cu biofilm concentration (µg Cu g^{-1} biofilm DW)	**Zn biofilm concentration (µg Zn g^{-1} biofilm DW)**
Initial	256 ± 104	4172 ± 1806
Control	438 ± 235	5360 ± 2034
Zn Control	302 ± 219	31823 ± 1619
1.4	163 ± 93	21954 ± 3901
5	342 ± 141	22460 ± 5345
80	1158 ± 358	23696 ± 5334
300	3397 ± 684	23322 ± 7067

Metal re-release from biofilm

The spiked biofilm was put in clean river water for the mayfly exposure and some re-release of metal species into the water occurred. The highest concentrations at 96 hours for Cu reached 62.6 µg L^{-1} and Zn reached 487 µg L^{-1} (Table 4.6). These values were equivalent to some of the lower dissolved exposure treatment concentrations.

Table 4.6: Mean ± 95% confidence intervals for dissolved water concentrations after the 96-hour mayfly exposure for the biofilm exposures (n=3)

	Treatment Cu (µg L^{-1})	Re-release of Cu (µg L^{-1})		Treatment Zn (µg L^{-1})	Re-release of Zn (µg L^{-1})
Cu only	0	1.1 ± 0.3	**Zn Only**	0	9.7 ± 4.8
	1.4	0.9 ± 0.3		8	6.9 ± 1.6
	5	1.1 ± 0.1		50	7.6 ± 1.8
	80	9.0 ± 1.6		150	9.6 ± 0.9
	300	26.4 ± 2.3		1500	113 ± 11.6
	1000	62.6 ± 3.0		5000	487 ± 61.4
Cu & Zn	0	0.8 ± 0.2		0	16.7 ± 6.3
	0	0.7 ± 0.0		1500	101 ± 13.4
	1.4	0.9 ± 0.1		1500	95.9 ± 25.6
	5	1.4 ± 0.2		1500	85.5 ± 9.4
	80	10.6 ± 1.2		1500	85.0 ± 10.4
	300	34.5 ± 0.7		1500	91.1 ± 4.0

4.3.3 Mayfly accumulation of metals from water and biofilm exposures

Copper

For both the water and biofilm, there were no significant difference in mayfly Cu concentrations between control, 1.4 and 5 µg L^{-1} (Figure 4.1). At 80 µg L^{-1} and above, mayfly Cu concentrations were significantly higher than the control (two-way ANOVA, $F_{5, 72}$ = 136.0, p <0.01) (TukeyHSD, p <0.01). There was an overall significant difference in mayfly Cu concentrations between the biofilm and water exposure (two-way ANOVA, $F_{1, 72}$ = 11.0, p <0.01). Mayfly Cu concentrations did not change significantly above 80 µg L^{-1} for the water exposure, however, Cu concentrations in the biofilm exposure were significantly higher at 300 and 1000 µg L^{-1} (TukeyHSD, p <0.01) (Figure 4.1).

Zinc

There was no difference in mayfly Zn concentration between biofilm and water exposures (two-way ANOVA, $F_{1, 72}$ = 0.7, n.s) (Figure 4.2). However, there was a significant difference in mayfly Zn concentrations between treatments (two-way ANOVA, $F_{5, 72}$ = 23.4, p <0.01). The 1500 µg L^{-1} treatment was significantly higher than the control, 8, and 50 µg L^{-1} treatments (TukeyHSD, p <0.01), while the highest treatment (5000 µg L^{-1}) had significantly higher mayfly Zn concentrations to all other treatments (TukeyHSD, p <0.01).

Cu and Zn mixture

There were no significant differences in mayfly Cu concentrations between the control, Zn control, 1.4, and 5 µg L^{-1} treatments (Figure 4.3). Copper accumulation was significantly higher in the tissue of mayflies for both the water and biofilm exposures at 80 µg L^{-1} (TukeyHSD, p <0.01). No overall significant difference was found in mayfly Cu accumulation between the biofilm and water exposures. However, for the 300 µg L^{-1} treatment on its own, the biofilm exposure had significantly higher mayfly Cu concentrations than the water exposure (TukeyHSD, p <0.05).

The treatments for the Cu and Zn mixture all had Zn concentrations of 1500 µg L^{-1}. Accumulation of Zn in the mayfly did not significantly increase within the biofilm or water exposure (Figure 4.4). The water exposure had significantly higher Zn concentrations than the biofilm control (TukeyHSD, p <0.05). However, no significant difference in mayfly Zn concentrations were found between the biofilm and water exposures at the same treatment levels.

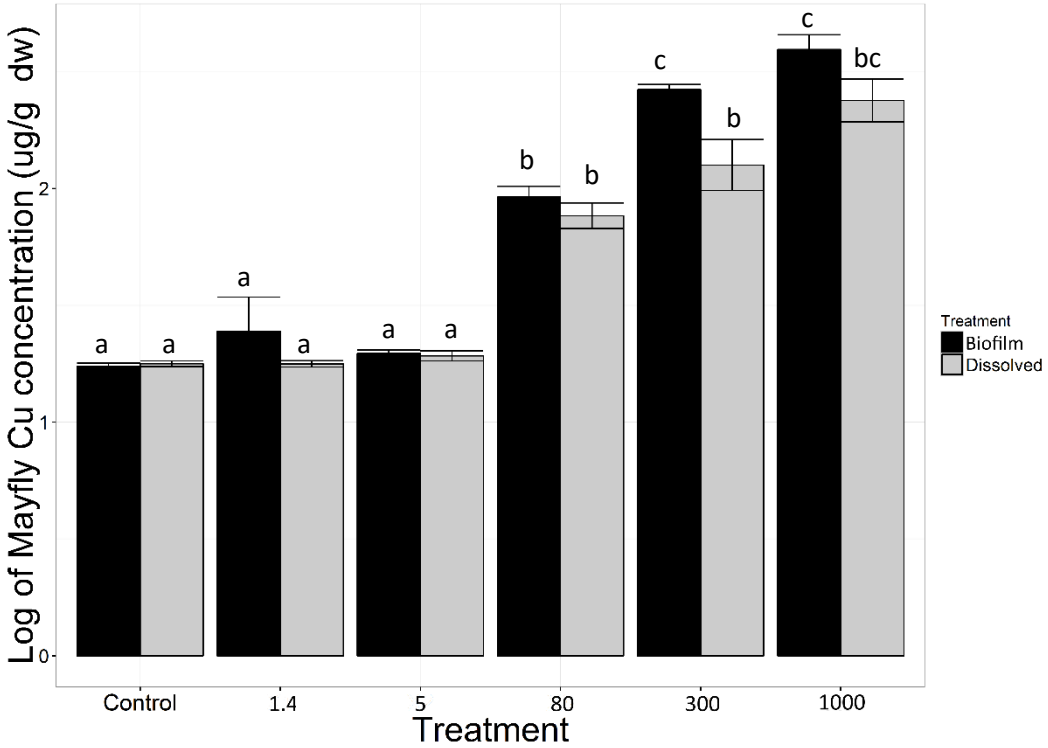

Figure 4.1: Mean (± 1 S.E.) whole body Cu concentration in mayflies for Cu-only experiment (n=7).

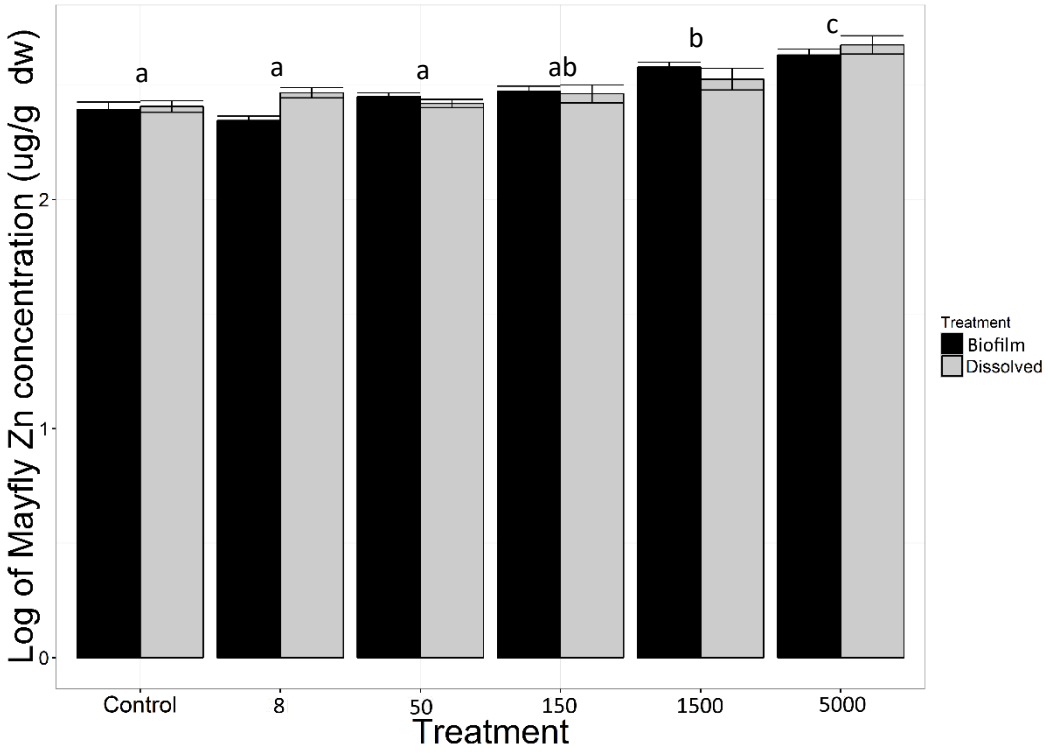

Figure 4.2: Mean (± 1 S.E.) whole body Zn concentrations of mayflies from Zn-only experiment (n=7).

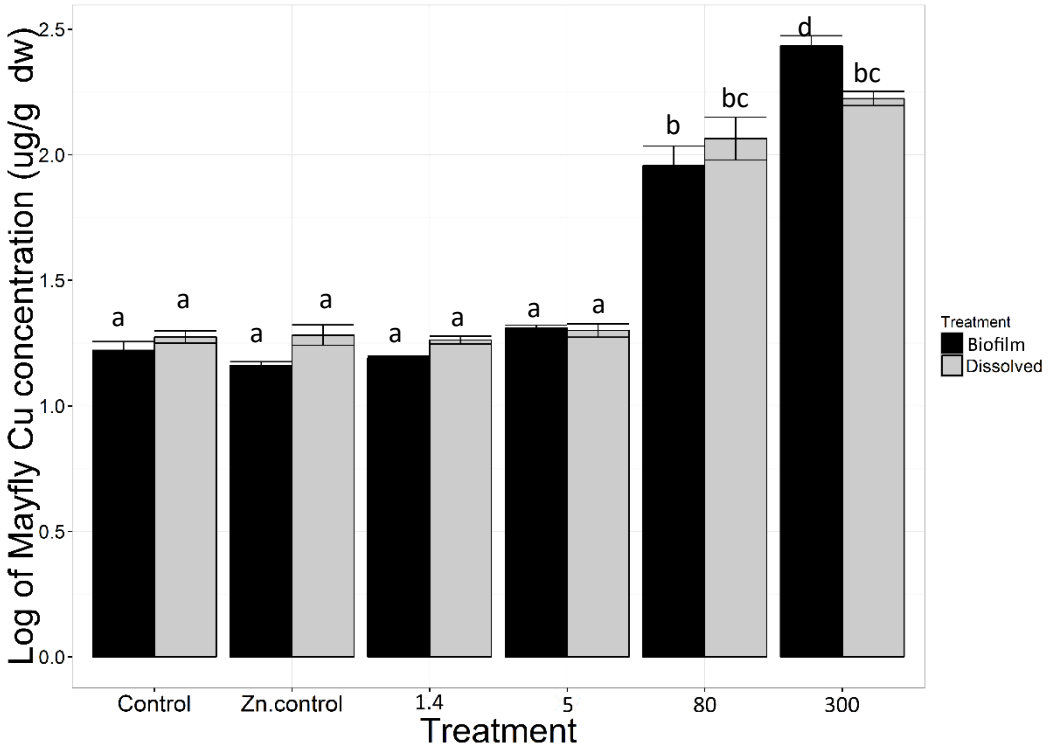

Figure 4.3: Mean (± 1 S.E.) whole body Cu concentrations of mayflies in combined Cu and Zn experiment (n=7).

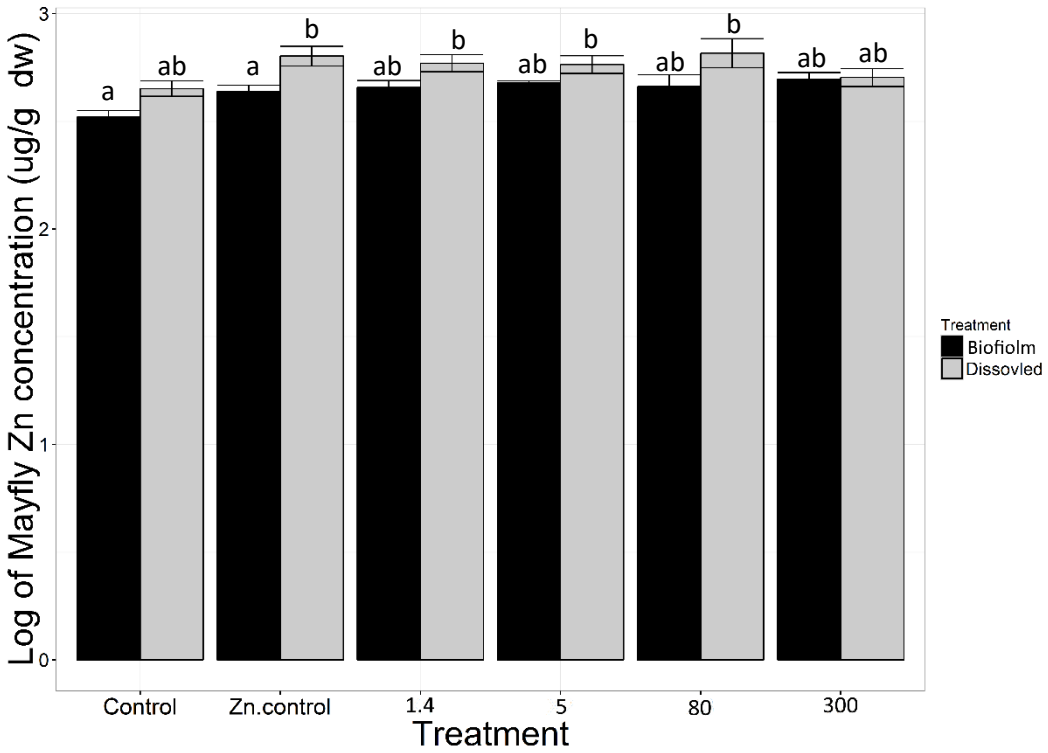

Figure 4.4: Mean (± S.E.) whole body Zn concentration of mayflies from combined Cu and Zn experiment (n=7).

4.3.4 Single metal and mixture comparison

There were no significant differences in Cu or Zn concentrations between experiments for the final biofilm metal concentrations, water metal concentrations, or initial whole tissue concentrations of the mayfly. Therefore, the concentration of bioaccumulation in the whole tissue of the mayfly can be compared between the metal mixture and single metal experiments.

This comparison showed that there were no significant differences for the mean concentration of Cu and Zn in the mayflies between the single metal and metal mixture experiments. This indicates that there is no difference in uptake by the *Deleatidium* mayfly with Cu and Zn alone or as a mixture.

4.3.5 Mortality

The water Cu exposure significantly increased in mortality at 1000 µg L^{-1} (two-way ANOVA, $F_{5, 72} = 3.1$, $p < 0.05$) (Figure 4.5). Mortality was random and relatively consistent for all other experiments and no other significant differences were present.

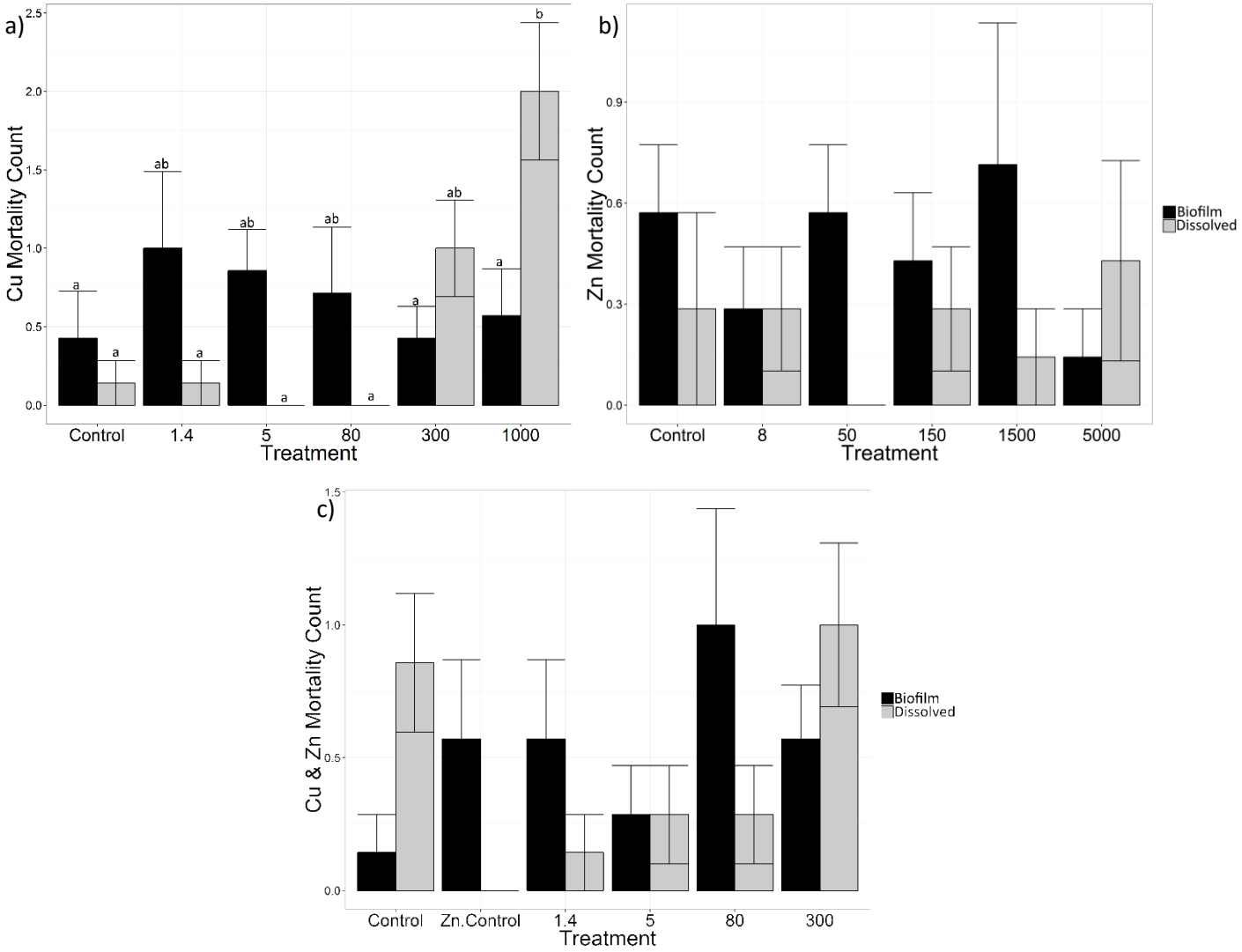

Figure 4.5: Mean ± 1 S.E. for mortality of mayflies in a) Cu, b) Zn, and c) Cu and Zn mixture experiments (n=7).

4.4 Discussion

4.4.1 Biofilm

Initial concentrations of the biofilm ranged from 4,171 – 4,661 and 256 – 367 µg g^{-1} for Zn and Cu respectively. These concentrations are at the higher end of those recorded in literature for natural streams. New Zealand studies have reported upper concentrations of biofilm Cu at 200 – 300 µg g^{-1} and 3,400 up to 7,100 µg g^{-1} for Zn (Golder Associates 2012; Ancion *et al.* 2013). These higher concentrations were recorded at sites with high urbanisation. Although the concentrations of metals in biofilm found at Cust Main Drain were high, the mayfly *Deleatidium* was abundant at the site and dissolved water concentrations for Cu and Zn were below ANZECC guidelines.

In this study, the accumulation of the associated metal in biofilm did not change significantly until the nominal concentrations of Zn at 1500 µg L^{-1} and Cu at 80 µg L^{-1}. Numerous reports show that biofilm in the environment concentrates metals from the water and often to greater concentrations than in sediments (Meylan *et al.* 2003; Ancion *et al.* 2010; Bradac *et al.* 2010). However, the point at which biofilm in this study showed significant uptake of the metal(s), was at concentrations generally higher than those reported in the environment in other studies. Ancion *et al.* (2010) found that maximal accumulation occurred during the first day of exposure, however, steady state concentrations between the water and biofilm did not occur until at least seven days. The biofilm in this study, would have accumulated a significant proportion of the metal(s) available, however, the high initial concentrations of the biofilm could have resulted in limited changes observed for the low treatment concentrations.

There was some large error associated with the biofilm metal concentrations, particularly at the higher concentrations, and this could be largely attributed to the selection of the cobbles for the experiment. Although it was attempted to select cobbles of similar size and biofilm coverage, there was some variation, resulting in variation for quantifying the amount of biofilm and concentration of the trace metals.

4.4.2 Mayflies

Copper

The concentrations of Cu in the mayfly were within the range reported for other benthic invertebrates (Beltman *et al.* 1999; Solà and Prat 2006; Duran *et al.* 2007). The highest mayfly Cu concentration measured in this study was 418 µg g^{-1}DW. This concentration is higher than has been reported in much of the literature, however, Beltman *et al.* (1999) reported concentrations up to 3,020 µg g^{-1} DW downstream of a mining site. Furthermore, benthic invertebrates that are grazers, such as the *Deleatidium* spp., are among the strongest accumulators of metals due to their feeding habits (Baudin and Fritsch 1989; Kiffney and Clements 1993).

The results from this study indicate that Cu accumulation in the mayfly was greater from biofilm exposure at the higher concentrations, however, no difference occurred at environmentally relevant concentrations. Significant accumulation of Cu in the mayfly tissue began at 80 µg L^{-1} for both the biofilm and water exposure. However, the average for the biofilm exposure at 1000 µg L^{-1} was not significantly higher than the water exposure. This was surprising considering the increase in concentration of Cu in the biofilm was significantly higher at this treatment than for the treatment at 300 µg L^{-1} (12, 800 and 4,400 µg g^{-1} respectively), and was a much higher increase than between 300 µg L^{-1} and 80 µg L^{-1}. This may be the result of an outlier in the data. There was a significantly higher value in the water 1000 µg L^{-1} Cu treatment of 660 µg g^{-1} DW compared to the mean value at this

treatment (275 µg g^{-1} DW). The value was included in the analysis as a reasonably large variation has been reported in literature. However, without this replicate included, there is a significant difference between the biofilm and water exposure results. Another factor was that there appeared to be a decrease in feeding activity by the mayflies beginning at the 300 µg L^{-1} treatment. The consumption of biofilm was not measured, but was observed. This could also explain the observed non-significance between diet and water exposure at this highest concentration. Sofyan *et al.* (2006) also found that feeding activity reduced or ceased at higher concentrations of Cu for the water flea, *Ceriodaphnia dubia*, resulting in a decreased Cu body burden. They recorded this at 74.7 µg Cu g^{-1} algal DW, which is a much lower concentration than reported in this study (12,800 µg g^{-1} DW).

The uptake through the biofilm in this study is either equivalent to or more significant for Cu accumulation than through water exposure. Hare *et al.* (2003) reviewed literature on the uptake of metals (specifically Cu, Cd, Ni, and Pb) from a food source and water exposure, and generally found contrasting results to this study. The crustacean amphipod *Hyalella* feeds on sediments and sediment-associated microflora, as well as on biofilm growing on rocks, similar to the *Deleatidium* spp. mayfly. They concluded that for the *Hyalella* species, the main route of exposure was dissolved metals, though, food could still be important. It should also be noted that re-release of the metal species into the water column from the biofilm did occur in the biofilm exposure. This meant that at the higher concentrations, there may have been some combined effects from dissolved and biofilm exposure.

Zinc

Maximum zinc concentrations in the mayfly in this study were 657 µg g^{-1} DW. The initial concentrations (222 µg g^{-1} DW) were at the upper end of concentrations reported by Timmermans (1993) and Duran *et al.* (2007). However, it was well within the range as reported by other studies such as Schmidt *et al.* (2011), which also reported mayflies accumulating higher concentrations of Zn compared to caddisflies.

Zinc did not accumulate in the mayflies to the same degree as Cu, nor was there a significant difference between the biofilm and water exposures. The highest treatment (5000 µg L^{-1}) for Zn increased 1.4- and 1.5-fold for biofilm and water exposure respectively, compared to 29-fold for biofilm Cu and 19.3-fold for the water Cu exposure. The uptake of Zn has been found to be slow in other overseas grazer mayfly species (Brinkman and Johnston 2008). This may be an explanation for the lower bio-accumulation of Zn. The limited data on metal uptake by endemic New Zealand invertebrate species make comparisons to literature difficult and highlights the need for more research in this area.

Both Cu and Zn are essential ions required for metabolic processes in aquatic insects (Hare 1992). Zinc in particular is a key component of many enzymes, including carbonic anhydrase (Rainbow 2002).

Goodyear and McNeill (1999) analysed available literature on heavy metal (specifically Cd, Cu, Pb, and Zn) bioaccumulation for freshwater macroinvertebrates. They found that Zn appeared to be regulated regardless of concentrations in the waters (biofilm or food source was not analysed). Therefore, the more effective regulation of Zn compared to Cu could explain the lower accumulation rates of Zn in this study.

Metal mixture

There are few studies that have investigated the effects of metal-mixtures on invertebrates, and no studies could be found where metal mixtures are compared with a food source and water exposures (DeForest and Meyer 2015).The inclusion of Zn at 1500 µg L^{-1} in the metal mixture experiment did not have an effect on accumulation of Cu by the mayflies for either the water or biofilm exposure. Both the Cu-only and metal mixture experiment showed an increase in Cu whole body concentration at the treatment of nominal concentration 80 µg L^{-1} and a significant difference between biofilm and water exposure at the treatment with nominal concentration of 300 µg L^{-1}.

The Cu and Zn mixture did not significantly affect the uptake of Zn. There were no significant differences in Zn concentrations of the mayfly tissue between the Zn-only and metal mixture experiments. Zinc concentrations in whole body mayflies therefore, result in a range of 1.1- to 1.5 – fold increase in both the Zn-only and mixture experiments. It is often reported that metal mixtures are very toxic to benthic invertebrates (Kiffney and Clements 1994; Hickey and Golding 2002). These results are frequently from mesocosm studies that focus on invertebrate community effects. For example, Hickey and Golding (2002) performed a mesocosm study of dissolved Cu and Zn mixture on an invertebrate community and found high chronic sensitivity for the *Deleatidium* sp. with 100% decrease in abundance at the 'high' concentrations (Cu: 13 µg L^{-1}, Zn: 570 µg L^{-1}). However, there was no comparison of the effect with single metals and the mixture, nor of food source versus water exposure.

In terms of individual invertebrate studies, there are a small number of studies that have explored the effects of metal mixtures compared with single metal species on individual aquatic invertebrates that have produced a wide range of results (Shaw *et al.* 2006). Norwood *et al.* (2003) conducted a review of all published metal mixture data on aquatic biota. Copper-Zinc interactions were summarised as follows; 11 cases were 'less than additive', one 'strictly additive', and nine cases were shown to be 'more than additive', clearly demonstrating the range of results when it comes to metal mixtures. The species used as the test organism as well as the concentrations of the associated trace metals result in a range of outcomes making comparisons to other studies difficult (Norwood *et al.* 2003).

Furthermore, of the studies undertaken for metal accumulation and metal mixtures, aquatic insects (such as the mayfly) are largely underrepresented (Cain *et al.* 2011).

Accumulation and toxicity

Mortality of the mayflies was only significant for the highest treatment in the Cu water exposure. There are a number of factors that affect the toxicity of trace metals to invertebrates, exploring the route of exposure and accumulation is only one component. Accumulation of a metal in the tissue of the organism does not necessarily mean it will produce toxic effects (Rainbow 2007). A metal can be stored as a non-toxic species or bound to metallothioneine (Gerhardt 1993). In addition to this, the toxicity depends on abiotic (e.g. pH, temperature) and biotic (e.g. organism size, life stage, tolerance) factors (Gerhardt 1993). The importance of life stage has often been identified as a reason for discrepancies in toxicity tests. Kim *et al.* (2012) demonstrated this in a study where the larvae of the mayfly *Centroptilum triangulifer* concentrated Zn 19-, 16-, and 17- fold compared to adults from the same cohort concentrating 8- , 3- , and 3- fold relative to the same dietary concentrations. It is also important to acknowledge that a high concentration in one species of mayfly may be low in another and comparisons of relative concentrations should be intraspecific (Rainbow 2002). This re-enforces the importance of data on benthic invertebrate species that are relevant to the area.

4.5 Conclusions

The biofilm significantly accumulated Cu at the 80 µg L^{-1} treatment. This was also when mayfly Cu concentrations significantly increased both in the biofilm and water exposure. This concentration was above what would be expected in the environment. However, over a more significant time period, accumulations at lower concentrations may be observed. Accumulation of Cu in the mayfly tissue was significantly higher for the biofilm exposure in the 300 µg L^{-1} treatment. Given that the accumulation from biofilm was either equal to or greater than the water exposure, this indicates that food sources may be a greater source of Cu than water exposure.

There were not many significant changes in the Zn accumulation in the mayflies for either the biofilm or water exposure. It may be that Zn is more regulated than Cu and therefore does not accumulate to the same degree. It also indicates that neither water or food source is more significant in accumulation of zinc in the *Deleatidium* spp. mayfly.

5 Chapter 5: Final conclusions and recommendations for future work

Increasing urbanisation places pressure on the ecological integrity of streams in many towns and cities (Walsh 2004; Paul and Meyer 2008). There is limited literature on the state of New Zealand's urban streams, particularly in terms of metal contamination and comparing different regions. Councils undertake monitoring programmes of their respective urban streams, however the monitoring and reporting process differs between urban centres. This thesis provided a study that compared the state of urban streams in three main urban centres of New Zealand and determined relationships between heavy metal contamination and the benthic invertebrate community.

Metal contamination was relatively consistent across New Zealand's three major urban centres. Once considering the relevant background concentrations for the sediment metals, only dissolved Cu was significantly different between the cities, with lower concentrations in Christchurch. Heavy metal concentrations generally increased together, allowing a sediment and water metal index to be determined from a PCA. The sediment metal index proved to be a significant variable in explaining the variation in the benthic invertebrate communities, although this contribution was relatively small.

This thesis supports the large amount of literature that identifies heavy metals as having some negative impacts on benthic invertebrates and therefore the ecological health of streams. However, the high significance of other environmental factors such as DO, indicate that additional factors associated with the urban stream syndrome are crucial to manage also. Strong relationships between taxa richness and mayfly richness with metal concentrations have been found in areas with high metal concentrations but low urbanisation (Hickey and Clements 1998). The degraded nature of urban streams makes it particularly difficult to establish links between cause and effect in urban ecosystems. The riparian and instream habitat, natural flow regimes and water and sediment quality all deteriorate with increased urbanisation (Pettigrove and Hoffmann 2003; Brown *et al.* 2009). The multiple factors contributing to the degradation in urban streams mean that management initiatives must be integrated, inclusive and catchment based (Walsh *et al.* 2005).

While it has been identified that the management of urban streams require consideration of all factors to be effective, this discussion focusses on the stormwater aspect as the focus of this thesis was on heavy metals. Under the Resource Management Act 1991, stormwater management and planning is primarily the responsibility of the associated regional council. The territorial authorities (city and district councils) must have a stormwater management plan in order to meet the requirements of discharge consents for the contaminants in the associated stormwater. Both territorial authorities and

regional councils undertake monitoring of stormwater quality. The accuracy and robustness of the council's monitoring approach is crucial to their ability to effectively manage the contaminants in stormwater. Inadequate or limited data may not provide an accurate report of the levels of contaminants in stormwater both in the catchment as a whole and specific sites.

The main approach to monitoring by councils is through measuring total and dissolved metal concentrations in the water column. This may not provide the most useful information on the level of contamination in urban streams. Sediment metal concentrations have a significant relationship with the benthic invertebrate community in this study, which has also been suggested in other literature (Kiffney and Clements 1993; Perdikaki and Mason 1999). This indicates that monitoring dissolved metals in the water column is not particularly useful in determining the ecological health of urban streams. Therefore, it is recommended that sediment sampling for metal concentrations be an integral part of monitoring and stormwater management plans.

Furthermore, much of the literature suggest that toxicity and uptake of metals are more important from the diet rather than water or sediment exposure (Rainbow 2002; Kim *et al.* 2012). This thesis found that this may be the case for Cu in the mayfly *Deleatidium* spp., however, diet may not be as important for Zn. It would be interesting to conduct a survey of metal contamination in food sources in conjunction with invertebrate sampling, as done in this thesis. However, this brings about many issues including determining what food source to sample, given the differing feeding characteristics of benthic invertebrates. It may be more useful to determine relationships between sediment metal concentrations and the differing food sources metal concentrations. It is also important to determine these relationships for each individual metal, as they do not all exhibit the same pattern. Therefore, the lowest concentration in the sediment (or water) that results in chronic effects from diet or habitat exposure must be determined. This could potentially provide better information for councils and result in more effective monitoring programmes.

Improving the robustness of council stormwater monitoring to include regular sediment sampling for metal concentrations (and determining relationships between sediment metal concentrations and the differing food sources metal concentrations) will enable more accurate relationships to be made between the discharge levels of metals and the adverse effects on the health of the waterways. This will result in improved stormwater management with more effective mitigation of these metal contaminants

There are little, if any, streams in urban centres that provide spaces with adequate stream health for mahinga kai practices (Pauling 2007). A major food source for many mahinga kai species are insects. A significant number of insects are included in the sensitive EPT taxa. If the stream cannot support the

life-capacity of the wide ranging insect taxa, then there will not be adequate adult insects in the area for the mahinga kai species to feed on. This degradation of the mauri (life force) of urban waterways is a major concern to tangata whenua. It adversely impacts on their ability for mahinga kai and undermines their role as kaitiaki of these waterways.

The results of this study provide the relevant iwi and rūnanga with information that may help in the protection and restoration of their freshwater streams. The improvements for monitoring identified above will help contribute towards protecting mahinga kai and kaitiakitanga values. Further research is needed that specifically examines the effects of contamination of stormwater discharges on tangata whenua cultural values; in particular, the abundance and diversity of freshwater mahinga kai species, the impacts on mahinga kai species consuming the insects and the consequent effects on human health eating the mahinga kai species.

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Appendix 1 – Site information

Table 6.0.1: Name, site code and GPS coordinates of sites in Auckland, Christchurch, and Wellington.

	Site Code	Site Name	Northing	Easting
Auckland	A1	Puhinui stream upper	5902829	1769424
	A2	Puhinui stream lowe	5904296	1766440
	A3	Otaki creek	5907450	1763987
	A4	Omaru creek	5917239	1765524
	A5	Meola creek	5917566	1753569
	A6	Lucas creek	5934510	1751468
	A7	Hillcrest stream	5929014	1751105
	A8	Kaipatiki stream	5925814	1753005
	A9	Swanson stream	5919321	1741227
	A10	Oakley creek	5917392	1751926
Christchurch	C1	Linwood canal	5178992	1574516
	C2	Smith st @ Matlock st	5178713	1573491
	C3	Okeover stream	5181009	1566621
	C4	Addington Brook	5179972	1569498
	C5	St Albans stream	5182687	1570619
	C6	Waimairi stream	5181189	1566987
	C7	Wairarapa stream	5181938	1567150
	C8	Smacks creek	5187925	1566848
	C9	Ballintines stream	5176125	1567682
	C10	Ilam stream	5180694	1567411
Wellington	W1	Owhiro bay	5421589	1747277
	W2	Brooklyn Central Park	5426401	1747904
	W3	Karori stream	5426477	1746925
	W4	Karori @ Makara MTB park	5427287	1744186
	W5	Porirua stream upper	5438726	1753367
	W6	Porirua lower @ Kenepuru stn	5443047	1754380
	W7	Cannons creek	5444529	1755148
	W8	Woburn rd	5435388	1759641
	W9	Speedy stream	5438187	1761678
	W10	Waiwhetu stream	5436586	1762779

Table 6.0.2: Physical habitat results for each site

Site	Surrounding land use	Riparian cover	Shading	Bank cover	Macrophytes
Upper Puhinui @ totara park (A1)	Reserve	TLB: soil, shrubs, native trees TRB: soil, shrubs, native trees	Heavily shaded	TLB: soil, trees TRB: soil, trees	None
Lower Puhinui @ plunket ave (A2)	Residential, commercial	TLB: grass, artificial TRB: grass, artificial	Open	TLB: artificial TRB: artificial	Submerged
Otaki creek @ golf course (A3)	residential, golf course, hospital	TLB: shrubs, soil, grass TRB: trees, shrubs, soil	Open	TLB: soil, grass TRB: soil, grass	Submerged
Omaru creek (A4)	Residential, park	TLB: soil, grass, native trees TRB: soil, grass, native trees	Open	TLB: soil, grass TRB: soil, grass	Submerged
Meola creek (A5)	Residential	TLB: soil, grass TRB: soil, grass	Partial	TLB: soil, grass TRB: soil, grass	Emergent
Lucas creek (A6)	Road, residential, reserve	TLB: grass, native trees, shrubs TRB: grass, native trees, shrubs	Partial	TLB: grass, shrubs, trees TRB: grass, shrubs, trees	None
Hillcrest stream (A7)	Road, residential	TLB: grass TRB: grass, concrete	Open	TLB: artificial TRB: artificial	None
Kaipatiki stream (A8)	Residential, Park	TLB: shrubs, trees TRB: shrubs, trees	Heavily shaded	TLB: soil, shrubs TRB: soil, shrubs	None
Swanson stream (A9)	Residential, park	TLB: grass, shrubs, native trees TRB: grass, shrubs, native trees	Heavily shaded	TLB: grass, shrubs TRB: grass, shrubs	None
Oakley creek (A10)	Residential, park, commercial	TLB: grass, shrubs, native trees TRB: grass, shrubs, native trees	Partial	TLB: grass, shrubs TRB: grass, shrubs	None
Linwood Canal @ Hargood st (C1)	Road, Residential	TLB: grass, soil, concrete TRB: concrete	Open	TLB: Artificial TRB: Artificial	Emergent
Smith St (C2)	Reserve	TLB: grass, shrubs, tussock TRB: grass, shrubs, tussock	Partial	TLB: Grass, Tussock TRB: grass, tussock	Marginal
Okeover stream @ University (C3)	Commercial, Residential	TLB: grass TRB: grass, tussock, shrubs	Partial	TLB: Grass TRB: grass, shrubs, trees	Submerged
Addington Brook (C4)	Hagley park	TLB: soil, ferns, native trees, tussock TRB: soil, ferns, native trees, tussock	Heavily shaded	TLB: soil, stony, tussock, trees TRB: soil, stony, tussock, trees	Marginal
St Albans Stream @ English Park (C5)	car park, Sports ground	TLB: grass, soil, concrete TRB: shrubs, grass	Heavily shaded	TLB: grass, soil TRB: soil	None
Waimairi stream @Fendalton park (C6)	Park, residential	TLB: grass, trees, tussock TRB: grass road	Partial	TLB: soil, tussock TRB: grass, soil	Submerged
Wairarapa stream @ Waiwetu reserve (C7)	Park, residential	TLB: grass, trees TRB: grass, trees, road	Heavily shaded	TLB: soil, tussock TRB: grass, soil	Submerged

Smacks creek @ reserve (C8)	reserve, road, timber mill	TLB: shrubs, native trees TRB: shrubs, native trees	Heavily shaded	TLB: soil, shrubs, trees TRB: soil, shrubs, trees	Emergent
Ballintines stream @ Sparks rd (C9)	rural farming, residential, road	TLB: soil TRB: soil	Open	TLB: Artificial TRB: Artificial	Submerged
Ilam stream @ Deans Bush (C10)	reserve, residential	TLB: grass, trees, tussock TRB: grass road	Partial	TLB: soil, tussock TRB: grass, soil	None
Owhiro bay (W1)	Residential, road	TLB: soil, trees TRB: soil, trees, grass	Heavily shaded	TLB: soil, stony TRB: soil, stony	Marginal
Brooklyn central park (W2)	Reserve/park	TLB: native trees, shrubs, tussock TRB: native trees, shrubs, tussock	Heavily shaded	TLB: soil, shrubs TRB: soil, shrubs	Marginal
Karori stream @ (W3)	Reserve, residential	TLB: ferns, native trees TRB: ferns, native trees	Heavily shaded	TLB: soil, trees TRB: soil, trees	None
Makara MTB park (W4)	Residential, road	TLB: soil, grass, concrete TRB: soil, grass	Heavily shaded	TLB: Artificial TRB: Artificial	Marginal
Upper Porirua stream (W5)	Residential	TLB: native trees, shrubs TRB: native trees, shrubs	Heavily shaded	TLB: soil, grass TRB: soil, grass	Submerged
Porirua stream @ kenepuru stn (W6)	Industry, commercial, road	TLB: soil, grass TRB: soil, grass	Open	TLB: soil, grass TRB: soil, grass	Marginal
Cannons stream (W7)	Residential, road, park	TLB: concrete, grass TRB: grass, soil	Open	TLB: soil, grass TRB: soil, grass	None
Woburn rd (W8)	Residential, park	TLB: soil, grass, rock TRB: soil, grass	Partial	TLB: soil, grass TRB: soil, grass, artificial	Marginal
Speedys creek (W9)	Park, road, school	TLB: grass, native trees TRB: grass, native trees	Partial	TLB: native trees, grass TRB: soil, grass	Marginal
Waiwhetu (W10)	Park, residential	TLB: soil, grass TRB: grass, native trees, ferns	Open	TLB: soil, grass TRB: grass, native trees	Marginal

Table 6..3: Percent (%) impervious area for each site. Data is extracted from the FENZ dataset

Site	% Impervious	Site	% Impervious	Site	% Impervious
A1	22.3	**C1**	91.0	**W1**	16.0
A2	45.6	**C2**	88.0	**W2**	70.1
A3	86.7	**C3**	15.0	**W3**	38.6
A4	82.6	**C4**	67.0	**W4**	67.3
A5	91.2	**C5**	96.0	**W5**	33.7
A6	32.5	**C6**	24.0	**W6**	33.9
A7	31.4	**C7**	69.0	**W7**	35.1
A8	64.4	**C8**	53.0	**W8**	89.5
A9	13.6	**C9**	76.0	**W9**	43.9
A10	82.5	**C10**	66.0	**W10**	41.5

Table 6.4: Physical and chemical parameters for the 30 sites sampled in three cities between March and June 2015. Data is based on a single sampling occasion. SI = substrate index.

Site	SI	Depth (m)	Width (m)	Velocity ($m\ s^{-1}$)	pH	Specific Conductivity ($\mu S_{25}\ cm^{-1}$)	Temperature (°C)	DO ($mg\ L^{-1}$)
A1	5.6	0.15	2.25	0.19	6.3	212	8.9	7.6
A2	2.0	0.40	1.8	0.04	6.1	174	16.0	6.7
A3	2.0	0.34	2.5	0.02	6.7	905	13.8	7.0
A4	3.7	0.11	1.1	0.32	6.8	296	12.3	7.1
A5	2.0	0.21	3.2	0.38	6.7	201	16.3	6.6
A6	4.1	0.22	2.3	0.09	6.9	270	10.9	7.1
A7	0.0	0.03	0.8	0.73	6.6	259	13.3	6.8
A8	3.7	0.10	0.93	0.25	7.8	211	11.5	7.0
A9	4.2	0.24	2.8	0.35	9.4	207	11.1	7.1
A10	4.4	0.33	2.5	0.19	7.7	370	12.0	6.9
C1	2.0	0.28	2.55	0.00	6.8	191	15.9	10.0
C2	2.9	0.33	2.5	0.00	6.5	145	15.0	5.1
C3	4.6	0.08	3.4	0.26	6.7	182	14.1	9.0
C4	5.1	0.15	1.3	0.11	7.4	270	13.5	7.4
C5	4.8	0.04	1	0.31	7.1	121	9.4	7.7
C6	4.9	0.09	2.25	0.20	7.2	176	13.8	9.4
C7	4.2	0.19	5	0.15	6.8	133	12.6	3.8
C8	4.6	0.15	1.72	0.46	7.1	106	14.3	6.2
C9	4.6	0.09	1.8	0.42	7.3	250	13.3	7.9
C10	5.2	0.20	3.8	0.48	6.7	190	13.8	8.7
W1	5.5	0.09	4.2	0.53	8.2	300	9.5	10.7
W2	5.1	0.04	1	0.30	7.9	203	8.5	11.2
W3	4.9	0.04	1.13	0.30	7.9	325	8.3	11.3
W4	5.4	0.11	4.1	0.32	7.5	193	10.1	11.1
W5	5.6	0.14	3.5	0.65	7.4	219	8.8	11.7
W6	5.5	0.21	6.2	0.69	7.3	219	9.7	11.5
W7	5.5	0.10	3.6	0.19	7.1	261	7.7	11.9
W8	5.1	0.16	1.8	0.10	6.9	238	9.7	5.0
W9	5.0	0.06	1.2	0.13	7.2	220	7.1	12.0
W10	4.7	0.10	2.3	0.36	7.4	237	8.8	11.4

Appendix 2 – Heavy metal concentrations

Table 7.0.1: Full water metals dataset for each site. T = total, F = filtered samples (µg L-1).

Site	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Sb	V	Zn
A1T	BDL	41	0.4	BDL	0.1	0.3	0.8	299	23.5	1	0.1	0.1	0.4	3
A1F	BDL	28	0.4	BDL	0.1	0.3	0.7	177	17.8	1	0.1	BDL	0.3	3
A2T	BDL	66	0.3	BDL	0.1	0.2	0.7	199	7.1	BDL	0.3	0.1	0.9	68
A2F	BDL	25	0.3	BDL	0.1	0.1	0.6	110	7.4	BDL	0.1	0.1	0.7	57
A3T	BDL	57	0.8	BDL	0.5	0.3	1.5	912	124.1	1	0.6	0.1	1.0	51
A3F	BDL	9	0.5	BDL	0.5	0.2	1.1	283	119.3	1	0.1	0.1	0.7	46
A4T	BDL	26	0.7	BDL	0.2	0.2	2.0	471	46.9	1	0.3	0.2	1.0	146
A4F	BDL	9	0.5	BDL	0.2	0.2	1.6	162	48.0	1	0.1	0.2	0.8	141
A5T	BDL	22	0.4	BDL	0.1	0.7	1.0	115	6.6	BDL	0.3	0.1	4.0	11
A5F	BDL	6	0.4	BDL	0.1	0.7	0.9	48	5.5	BDL	0.1	0.1	3.9	12
A6T	BDL	50	0.7	BDL	0.3	0.5	1.1	825	46.9	2	0.1	0.1	0.8	6
A6F	BDL	19	0.5	BDL	0.3	0.5	0.9	406	47.1	2	0.1	0.1	0.6	6
A7T	BDL	47	0.9	BDL	0.3	0.4	1.2	752	23.5	2	0.2	0.1	0.6	33
A7F	BDL	22	0.7	BDL	0.3	0.4	1.1	356	23.9	1	0.1	0.1	0.5	23
A8T	BDL	96	0.5	BDL	0.7	0.6	1.7	707	29.5	3	0.6	0.1	0.8	21
A8F	BDL	27	0.3	BDL	0.6	0.5	1.3	334	25.9	3	0.2	0.1	0.4	18
A9T	BDL	108	0.3	BDL	0.4	0.3	1.2	959	54.3	1	0.3	0.1	0.9	12
A9F	BDL	41	0.2	BDL	0.4	0.2	0.9	436	49.9	1	0.1	0.1	0.5	6
A10T	BDL	107	0.8	BDL	0.4	0.8	2.3	560	51.1	2	1.3	0.5	2.3	26
A10F	BDL	23	0.6	BDL	0.3	0.5	1.6	188	37.3	2	0.2	0.5	1.7	18
C1T	BDL	29	0.9	BDL	BDL	0.1	0.2	328	11.5	BDL	0.3	0.1	0.2	7
C1F	BDL	21	0.7	BDL	BDL	0.1	0.2	109	14.0	BDL	0.1	0.1	0.2	3
C2T	BDL	170	1.6	BDL	0.2	0.5	2.3	263	19.2	BDL	1.9	0.3	0.7	35
C2F	BDL	50	2.3	BDL	0.1	0.5	2.9	89	25.2	BDL	0.6	0.5	0.9	44

C3T	BDL	12	0.1	BDL	BDL	0.1	1.8	19	0.4	BDL	0.1	BDL	0.2	5
C3F	BDL	8	0.1	BDL	BDL	0.1	0.4	17	0.4	BDL	0.1	BDL	0.2	4
C4T	BDL	15	1.0	BDL	0.1	0.1	0.5	245	34.1	BDL	0.2	0.3	0.1	12
C4F	BDL	6	1.2	BDL	0.1	0.1	0.8	167	76.9	BDL	0.1	0.4	0.2	19
C5T	BDL	81	3.0	BDL	0.1	0.4	1.9	270	25.4	1	4.9	0.2	1.4	35
C5F	BDL	8	2.7	BDL	0.1	0.2	0.8	141	22.6	1	1.6	0.2	1.3	22
C6T	BDL	8	0.4	BDL	BDL	0.1	0.3	36	2.4	BDL	0.2	0.1	0.3	5
C6F	BDL	2	0.4	BDL	BDL	0.1	0.2	25	2.2	BDL	0.1	0.1	0.3	5
C7T	BDL	2	0.1	BDL	BDL	BDL	0.1	8	1.1	BDL	0.1	BDL	BDL	1
C7F	BDL	1	0.1	BDL	BDL	BDL	0.1	11	2.1	BDL	BDL	BDL	0.1	3
C8T	BDL	6	2.0	BDL	BDL	1.4	0.3	18	2.8	BDL	BDL	0.1	0.3	1
C8F	BDL	2	1.9	BDL	BDL	1.4	0.3	11	2.6	BDL	BDL	0.1	0.3	1
C9T	BDL	2	0.2	BDL	BDL	BDL	0.1	24	1.1	BDL	BDL	BDL	0.1	2
C9F	BDL	2	0.1	BDL	BDL	BDL	0.0	15	2.3	BDL	BDL	BDL	0.1	2
C10T	BDL	2	0.2	BDL	BDL	0.1	0.3	18	1.2	BDL	0.1	BDL	0.1	5
C10F	BDL	3	0.1	BDL	BDL	BDL	0.2	12	2.4	BDL	0.1	BDL	0.2	2
W1T	BDL	12	0.8	BDL	0.3	0.5	1.3	145	141.1	BDL	0.2	0.3	0.3	7
W1F	BDL	6	0.7	BDL	0.3	0.4	1.2	54	197.1	BDL	0.1	0.2	0.3	7
W2T	BDL	14	0.9	BDL	BDL	0.2	2.4	31	1.9	BDL	0.2	0.2	0.5	14
W2F	BDL	11	0.9	BDL	BDL	0.2	2.3	26	2.2	BDL	0.2	0.2	0.5	15
W3T	BDL	6	0.6	BDL	BDL	0.1	0.6	19	0.2	BDL	0.1	0.1	0.4	4
W3F	BDL	4	0.5	BDL	BDL	0.1	0.5	17	0.2	BDL	0.1	0.1	0.4	2
W4T	BDL	17	0.5	BDL	BDL	0.3	2.2	50	1.8	BDL	0.2	0.1	0.3	57
W4F	BDL	13	0.5	BDL	0.1	0.2	2.1	52	17.4	BDL	0.2	0.1	0.3	54
W5T	BDL	30	0.5	BDL	BDL	0.2	1.1	97	3.0	BDL	0.2	0.1	0.4	10
W5F	BDL	17	0.5	BDL	0.1	0.2	1.0	66	8.2	BDL	0.1	0.1	0.4	11
W6T	BDL	40	0.5	BDL	BDL	0.2	1.4	159	11.3	BDL	0.2	0.1	0.4	16
W6F	BDL	16	0.5	BDL	0.1	0.2	1.2	88	24.8	BDL	0.1	0.1	0.4	15
W7T	BDL	83	0.6	BDL	0.3	0.2	1.1	520	116.9	BDL	0.2	0.1	0.4	9

W7F	BDL	23	0.5	BDL	0.3	0.2	0.9	305	136.6	BDL	0.1	0.1	0.3	7
W8T	BDL	6	1.1	BDL	0.1	0.1	3.9	505	30.6	1	0.5	0.2	0.2	120
W8F	BDL	4	1.0	BDL	0.3	0.2	1.9	408	95.0	1	0.3	0.2	0.2	123
W9T	BDL	57	0.3	BDL	BDL	0.2	1.1	132	2.8	BDL	0.2	0.1	0.5	4
W9F	BDL	36	0.3	BDL	BDL	0.2	1.0	97	4.9	BDL	0.1	0.1	0.5	4
W10T	BDL	37	0.7	BDL	0.2	0.2	1.4	443	28.3	1	0.2	0.1	0.3	17
W10F	BDL	18	0.7	BDL	0.6	0.2	1.0	369	64.0	1	0.1	0.1	0.2	16

BDL = below detection limits

Table 7.0.2: Heavy metal < 2mm sediment concentrations for each site where sediment samples were collected (mg kg-1). Sites A7, W2, and W6 had no sediment samples.

	Al	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Ag	Cd	Sb	Pb
A1	8059	18.8	10.7	457.0	13601	8.0	15	8.5	57	3.3	0.1	0.1	0.1	6.5
A2	12787	38.1	33.5	382.7	25978	20.5	107	61.5	286	4.9	2.8	0.3	0.4	87.2
A3	16344	59.4	32.0	340.3	26568	14.7	56	48.0	290	5.6	0.1	0.2	0.3	73.6
A4	12289	59.5	25.5	746.5	28886	19.7	44	18.8	346	5.3	0.2	0.1	0.1	23.6
A5	6647	31.4	26.1	220.9	13408	9.0	36	45.1	291	4.8	1.9	0.3	1.0	109.4
A6	13054	37.9	16.8	495.8	25404	15.8	50	22.8	90	4.1	0.1	0.1	0.1	12.8
A8	7906	21.0	12.2	65.3	7966	3.5	8	6.3	41	2.3	BDL	BDL	BDL	9.2
A9	7781	34.7	13.9	480.5	25373	25.1	35	38.0	98	6.6	0.1	0.1	0.7	32.2
A10	9254	36.8	21.1	384.3	23118	13.7	39	24.8	274	6.6	0.1	0.3	0.2	55.9
C1	7336	22.5	19.1	815.7	29885	7.2	11	30.8	869	19.8	0.1	0.3	1.2	75.7
C2	9435	20.5	14.6	213.4	14614	5.9	12	16.6	178	6.4	0.1	0.1	0.2	39.5
C3	5253	12.4	9.8	148.5	10514	3.5	7	21.6	80	2.6	0.1	BDL	0.2	52.1
C4	6146	16.8	14.5	435.2	19047	9.3	13	19.9	588	13.4	0.1	0.3	0.3	86.1
C5	5598	12.1	9.3	166.8	9738	4.1	8	8.9	161	2.8	0.1	0.1	0.1	35.2
C6	4549	10.4	8.4	116.5	8073	3.0	6	8.7	101	1.6	0.1	0.1	0.1	50.8
C7	5920	12.9	10.1	148.7	10541	3.4	8	8.2	84	1.3	0.1	0.1	0.1	24.5
C8	4937	11.1	15.5	120.6	8768	2.7	7	5.5	44	2.0	0.0	0.1	0.1	17.3
C9	5564	13.1	11.2	195.3	11472	6.1	9	11.4	157	5.7	0.1	0.1	0.2	40.0
C10	5890	12.3	10.1	144.7	9675	3.5	7	9.7	120	1.2	0.1	0.1	0.1	36.2
W1	9277	19.5	11.1	399.7	17042	9.1	11	18.0	335	5.2	0.1	0.2	0.3	106.1
W3	14725	29.3	20.4	393.7	21830	9.4	16	13.6	107	4.4	0.1	0.1	0.1	34.9
W4	11865	24.5	19.4	321.8	24624	8.3	14	32.6	286	12.9	0.1	0.5	0.2	111.3
W5	13332	26.5	17.5	411.1	21637	8.9	14	16.3	135	3.9	0.1	0.1	0.1	62.8
W7	8747	18.7	10.9	366.6	13591	6.0	9	8.5	114	2.9	0.1	0.1	0.1	21.1
W8	10853	23.2	18.6	247.1	18727	8.5	13	102.7	586	9.4	1.9	0.7	0.5	161.9
W9	11169	25.2	13.8	268.2	14616	5.6	8	12.4	117	3.2	0.1	0.1	0.2	29.8
W10	7993	16.7	12.5	230.3	15959	6.4	10	23.0	256	4.9	0.1	0.3	0.2	40.2

BDL = below detection limits

Table 7.0.3: Heavy metal < 63µm sediment concentrations for each site where this size fraction was obtainable (mg kg-1) Sites A7, W1, W3, and W6 did not have < 63µm sampels

	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Sb	Pb
A1	27.2	24.2	1029.1	19904	12.6	18	11.7	96	5.1	0.1	0.1	12.7
A2	64.4	46.2	434.2	31402	15.4	65	56.1	381	5.8	0.3	0.7	61.8
A3	70.7	49.8	345.9	35848	13.1	36	159.2	590	9.7	0.6	0.5	97.5
A4	56.4	27.6	674.4	24872	14.2	15	21.0	320	3.8	0.1	0.1	20.3
A5	47.0	37.6	319.5	20744	10.5	37	48.4	304	5.1	0.2	0.8	101.4
A7	57.9	19.6	608.6	28019	12.3	19	25.2	133	6.0	0.1	0.4	18.0
A8	24.3	10.0	68.5	8725	3.0	6	6.9	43	2.1	0.0	0.0	9.3
A9	40.5	33.4	414.8	53531	43.9	78	119.3	81	15.6	0.1	1.6	57.1
A10	44.1	32.2	533.7	20150	13.0	23	39.2	366	6.6	0.3	0.3	65.5
C1	33.3	27.3	1539.7	59713	9.1	14	39.5	1632	32.4	0.3	1.5	110.0
C2	22.8	18.0	264.9	19570	6.5	13	17.4	251	8.6	0.1	0.2	50.3
C3	11.0	9.9	145.9	9166	2.9	6	22.3	63	1.5	0.0	0.1	40.6
C4	17.9	19.8	635.3	22238	10.5	14	31.8	799	8.7	0.3	0.4	56.9
C5	13.9	14.0	236.0	13451	4.5	9	15.8	287	4.8	0.2	0.2	62.3
C6	12.1	12.1	177.1	12697	4.2	8	21.3	179	3.4	0.1	0.5	102.9
C7	12.0	11.9	162.7	12852	3.3	8	17.1	119	2.3	0.1	0.2	39.1
C8	9.8	22.3	134.8	10313	2.5	7	13.3	63	3.8	0.1	0.3	19.0
C9	14.2	24.2	319.6	16086	8.9	10	30.5	252	11.1	0.2	0.2	71.5
C10	9.3	9.2	131.2	9507	2.7	6	15.9	126	1.7	0.1	0.1	49.2
W2	24.5	13.3	574.0	21226	11.4	10	42.8	348	11.0	0.4	0.5	168.5
W4	26.1	21.8	390.3	28555	8.6	12	47.5	300	19.6	0.2	2.0	166.2
W5	23.1	13.1	425.0	16651	6.5	9	18.1	119	4.8	0.1	0.1	49.4
W7	16.6	8.6	395.4	11461	4.4	6	9.9	103	3.1	0.1	0.1	18.0
W8	20.7	25.8	318.5	24039	5.7	11	139.7	882	26.2	0.4	1.5	409.9
W9	22.0	11.7	276.1	13514	4.7	6	10.7	116	2.7	0.1	0.1	23.5
W10	13.7	12.4	258.1	15228	5.3	7	48.6	272	6.4	0.3	0.3	51.7

Table 7.0.4: Contamination factor (CF) for sediment heavy metals. Those in bold have low contamination, and those highlighted have intermediate contamination

Site	As	Cd	Cu	Ni	Pb	Zn
A1	0.27	0.08	0.19	0.44	0.10	0.32
A2	0.41	0.39	0.68	0.34	**1.34**	0.25
A3	0.46	0.37	0.53	0.18	**1.13**	0.25
A4	0.44	0.17	0.42	**1.26**	0.36	**1.92**
A5	0.40	0.43	0.50	0.11	**1.68**	0.25
A6	0.34	0.09	0.51	**1.43**	0.20	0.50
A7	0.00	0.00	0.00	0.00	0.00	0.00
A8	0.19	0.05	0.14	0.22	0.14	0.23
A9	0.55	0.20	0.85	**1.00**	0.50	0.55
A10	0.55	0.39	0.28	0.12	0.86	0.24
C1	**1.81**	**1.29**	**1.29**	0.67	**2.12**	6.15
C2	0.60	0.66	0.71	0.74	**1.13**	**1.29**
C3	0.17	0.20	**1.22**	0.43	0.52	0.54
C4	0.88	**1.56**	**1.12**	0.81	0.85	3.94
C5	0.19	0.55	0.50	0.46	0.35	**1.08**
C6	0.15	0.29	0.37	0.39	**1.46**	0.73
C7	0.08	0.38	0.47	0.47	0.24	0.56
C8	0.13	0.49	0.31	0.40	0.17	0.29
C9	0.54	0.56	0.49	0.57	**1.14**	**1.14**
C10	0.12	0.28	0.42	0.46	**1.04**	0.87
W1	0.00	0.00	0.00	0.00	0.00	0.00
W2	0.74	**1.86**	0.72	0.86	**1.35**	3.19
W3	0.63	**1.05**	0.54	**1.19**	0.44	**1.02**
W4	**1.85**	4.92	**1.31**	**1.06**	**1.42**	**2.72**
W5	0.55	0.76	0.65	**1.08**	0.80	**1.28**
W6	0.00	0.00	0.00	0.00	0.00	0.00
W7	0.42	0.55	0.34	0.66	0.27	**1.09**
W8	**1.40**	3.57	3.54	0.93	**2.20**	**2.93**
W9	0.45	0.44	0.65	0.60	0.41	0.58
W10	0.70	**1.67**	**1.21**	0.72	0.55	**1.27**

Appendix 3 – Benthic invertebrate presence/absence

Table 8.0.1: MCI and UCI tolerance scores and Auckland presence/absence of benthic invertebrates, 'y' indicates presence.

	MCI	UCI	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Ephemeroptera												
Coloburiscus	9	1.871								y		
Deleatidium	*8*	1.161										
Neozephlebia	*7*	1.89										
Nesameletus	*9*	1.92										
Zephlebia	*7*	1.89								y		
Plecoptera												
Acroperla	*5*	1.184						y		y	y	
Austroperla	*9*	2.052										
Cristperla	*8*	2.052										
Spaniocerca	*8*	2.052									y	
Stenoperla	*10*	0.923										
Zelandobius	*5*	1.728									y	
Trichoptera												
Aoteapsyche	*4*	1.358										
Diplectrona	*9*	9										
Hudsonema?	*6*	0.704	y							y		y
Hydrobiosis	*5*	0.989										
Triplectides?	*5*	0.72									y	
H. parumbripennis	*5*	0.989										
Oeconesus	*9*	0.119										
Oecetis	*6*	-0.772										
Olinga	*9*	2.073										
Orthopsyche	*9*	9								y		
Oxyethira	*2*	0.248						y	y			
Polyplectropus	*8*	0.145										
Pseudoeconesus	*9*	0.119										

Psilochorema	*8*	0.571										
Pycnocentria	*7*	1.462										
Pycnocentrodes	*5*	1.472										
Coleoptera												
Antiporus	*5*	-1.079										
Elmidae	*6*	1.515										
Liodessus	*5*	-0.601										
Scirtidae	*8*	0.624									y	
Diptera												
Austrosimulium	*3*	1.026					y				y	
Chironomus	1	-0.611	y	y					y			
Culex	*3*	-0.21										
Empididae	3	0.425										
Hexatomi	5	0.355										
Muscidae	3	0.025										
Orthoclad	2	0.438			y				y		y	y
Paradixa	*4*	-0.365										
Tanyderidae	4	0.964										
Tanypodinae	5	-0.797		y								
Tipulidae	5	5										
Megaloptera												
Archichauliodes diversus	*7*	1.729										
Odonata												
Austrolestes	*6*	-0.766										
Xanthconemis	*5*	0.35					y					y
Crustacea												
Amphipoda	5	-0.567	y	y			y	y				y
Cladoceran	*5*	-0.181										

Copepoda	*5*	0.077											
Ostracoda	3	-0.67			y		y		y				
koura	5	0.774											
Mollusca													
Gyraulus	*3*	-0.565		y			y		y		y		
Limnea	*3*	0.721											
Physa	*3*	-0.494	y	y			y	y	y		y	y	
Potamopyrgus	*4*	0.023	y		y	y	y	y	y	y	y	y	
Sphaerium	*3*	-0.612	y										
Oligochaeta	*1*	-0.277	y	y	y	y	y	y	y		y	y	
Nematode													
Flatworms													
Cura sp.	*3*	-0.29		y		y	y		y	y	y		
Hydra	3	-0.607		y									
Acari	*5*	0.132	y				y						
Microvelia	*5*	-0.169											
Collembola	6	-0.15	y	y					y		y		

Table 8.0.2: MCI and UCI tolerance scores and Christchurch presence/absence of benthic invertebrates, 'y' indicates presence.

	MCI	UCI	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Ephemeroptera												
Coloburiscus	9	1.871										
Deleatidium	*8*	1.161										
Neozephlebia	*7*	1.89										
Nesameletus	*9*	1.92										
Zephlebia	*7*	1.89										
Plecoptera												
Acroperla	*5*	1.184										
Austroperla	*9*	2.052										
Cristperla	*8*	2.052										
Spaniocerca	*8*	2.052										
Stenoperla	*10*	0.923										
Zelandobius	*5*	1.728										
Trichoptera												
Aoteapsyche	*4*	1.358										
Diplectrona	*9*	9										
Hudsonema?	*6*	0.704				y		y	y	y	y	y
Hydrobiosis	*5*	0.989										
Triplectides?	*5*	0.72			y	y		y	y	y		y
H. parumbripennis	*5*	0.989										y
Oeconesus	*9*	0.119			y			y				y
Oecetis	*6*	-0.772				y						
Olinga	*9*	2.073										
Orthopsyche	*9*	9										
Oxyethira	*2*	0.248					y		y	y		
Polyplectropus	*8*	0.145										
Pseudoeconesus	*9*	0.119										
Psilochorema	*8*	0.571						y		y		y
Pycnocentria	*7*	1.462			y			y				

Pycnocentrodes	*5*	1.472			y			y				
Coleoptera												
Antiporus	*5*	-1.079										
Elmidae	*6*	1.515										
Liodessus	*5*	-0.601										
Scirtidae	*8*	0.624										
Diptera												
Austrosimulium	*3*	1.026										
Chironomus	1	-0.611	y	y								
Culex	*3*	-0.21										
Empididae	3	0.425								y		y
Hexatomi	5	0.355										
Muscidae	3	0.025								y		
Orthoclad	2	0.438	y	y		y	y	y	y			
Paradixa	*4*	-0.365										
Tanyderidae	4	0.964										
Tanypodinae	5	-0.797					y	y	y			
Tipulidae	5	5										
Megaloptera												
Archichauliodes diversus	*7*	1.729										
Odonata												
Austrolestes	*6*	-0.766										
Xanthconemis	*5*	0.35	y	y								
Crustacea												
Amphipoda	5	-0.567			y			y	y	y	y	y
Cladoceran	*5*	-0.181	y	y			y	y	y			
Copepoda	*5*	0.077										
Ostracoda	3	-0.67	y	y		y	y	y	y	y	y	y

koura	5	0.774										
Mollusca												
Gyraulus	*3*	-0.565	y	y					y			
Limnea	*3*	0.721										
Physa	*3*	-0.494	y	y	y	y	y	y	y	y	y	y
Potamopyrgus	*4*	0.023	y	y	y	y	y	y	y	y	y	y
Sphaerium	*3*	-0.612	y	y		y	y	y	y	y	y	y
Oligochaeta	*1*	-0.277	y	y	y	y	y	y	y	y	y	y
Nematode												
Flatworms												
Cura sp.	*3*	-0.29	y		y	y	y					
Hydra	3	-0.607										
Acari	*5*	0.132										
Microvelia	*5*	-0.169	y			y						
Collembola	6	-0.15	y	y		y	y	y	y			

Table 8.0.3: MCI and UCI tolerance scores and Wellington presence/absence of benthic invertebrates, 'y' indicates presence.

	MCI	UCI	W1	W2	W3	W4	W5	W6	W7	W8	W9	W10
Ephemeroptera												
Coloburiscus	9	1.871				y	y				y	y
Deleatidium	*8*	1.161			y	y	y	y	y			y
Neozephlebia	*7*	1.89			y							
Nesameletus	*9*	1.92					y					
Zephlebia	*7*	1.89			y							
Plecoptera												
Acroperla	*5*	1.184										
Austroperla	*9*	2.052										y
Cristperla	*8*	2.052										
Spaniocerca	*8*	2.052		y	y		y	y				
Stenoperla	*10*	0.923			y							
Zelandobius	*5*	1.728										
Trichoptera												
Aoteapsyche	*4*	1.358	y				y	y	y		y	
Diplectrona	*9*	9	y	y	y							
Hudsonema?	*6*	0.704										y
Hydrobiosis	*5*	0.989					y		y			y
Triplectides?	*5*	0.72										
H. parumbripennis	*5*	0.989										
Oeconesus	*9*	0.119										y
Oecetis	*6*	-0.772										
Olinga	*9*	2.073										
Orthopsyche	*9*	9			y	y					y	y
Oxyethira	*2*	0.248										
Polyplectropus	*8*	0.145										
Pseudoeconesus	*9*	0.119									y	
Psilochorema	*8*	0.571			y		y				y	y
Pycnocentria	*7*	1.462							y			

Pycnocentrodes	*5*	1.472							y	y	y			
Coleoptera														
Antiporus	*5*	-1.079												
Elmidae	*6*	1.515							y	y	y			
Liodessus	*5*	-0.601												
Scirtidae	*8*	0.624												
Diptera														
Austrosimulium	*3*	1.026												
Chironomus	1	-0.611				y		y					y	y
Culex	*3*	-0.21												
Empididae	3	0.425												
Hexatomi	5	0.355				y			y					
Muscidae	3	0.025											y	y
Orthoclad	2	0.438	y	y	y	y				y	y	y		y
Paradixa	*4*	-0.365												
Tanyderidae	4	0.964												
Tanypodinae	5	-0.797								y		y		y
Tipulidae	5	5												
Megaloptera														
Archichauliodes diversus	*7*	1.729					y		y	y	y		y	y
Odonata														
Austrolestes	*6*	-0.766												
Xanthconemis	*5*	0.35												
Crustacea														
Amphipoda	5	-0.567	y	y					y	y	y	y	y	y
Cladoceran	*5*	-0.181												
Copepoda	*5*	0.077												
Ostracoda	3	-0.67								y		y		

koura	5	0.774				y								
Mollusca														
Gyraulus	*3*	-0.565										y		
Limnea	*3*	0.721												
Physa	*3*	-0.494					y					y		
Potamopyrgus	*4*	0.023	y			y	y	y	y	y	y	y	y	
Sphaerium	*3*	-0.612					y				y	y		
Oligochaeta	*1*	-0.277	y				y	y	y	y	y	y	y	
Nematode														
Flatworms														
Cura sp.	*3*	-0.29		y		y		y			y	y		y
Hydra	3	-0.607												
Acari	*5*	0.132										y		
Microvelia	*5*	-0.169							y					
Collembola	6	-0.15	y	y		y	y	y	y			y	y	y

Appendix 4 – Letter of support

Hoana Burgman and Clare Williams,
Tuahiwi Marae,
219 Tuahiwi Rd,
Tuahiwi 7691,
Kaiapoi,
North Canterbury.

24th May 2016

Malea Zygadlo,
307 Pine Ave,
South Brighton,
Christchurch 8062.

Tēnā koe Malea,

It is my pleasure to write a letter in support of your Master's thesis research to be submitted to Te Whare Wānanga o Waitaha (University of Canterbury) this year. Your research topic which investigates the impacts of heavy metals in urban streams (including Christchurch) on the benthic invertebrate community is highly relevant to our cultural values of kaitiakitanga and mahinga kai. Freshwater is a treasured taonga and its degraded mauri is a great concern to Te Ngāi Tūāhuriri Rūnanga.

We appreciate you discussing the topic with Te Ngāi Tūāhuriri Rūnanga at the beginning of your research at a hui held at Tauhiwi Marare in March, 2015. This process acknowledges us as mana whenua of the takiwā in which you will conduct sampling of the Christchurch urban streams. It also gave us the opportunity to provide information on the location of specific sites that are culturally significant to us.

I believe the project has potential value to help us in our understanding of the impacts heavy metals have on the mauri of our urban streams and the implications for mahinga kai.

Nāku, nā

Hoana Burgman
H.M. Burgman

Clare Williams
C M Williams

(Environmental and cultural advisors for Te Ngāi Tūāhuriri Rūnanga)

Appendix 5 – Site characterisation field sheet example

Appendix 4

P1 - Site characterization field sheet

Site	Site code	CM1	Site name	Maragood st	GPS	N -		
	Assessor		Date	20/4/15		E -		
Channel & Bank	Wetted channel width	m	Vegetated bank width	m	Site length	m	* Channel & bank notes	
	Channel shape	Artificially channelised ✓	Straight	Weakly sinuous	Strongly sinuous			
	Flow conditions	Low flow	Base flow ✓	High flow				
	Flow types present	Riffle/rapid ☐	Run ☐	Pool ☑	Other ☐			
	Lower bank height	L – m	R – m	Upper bank height	L – m	R – m		
	Bank stability	Stable ✓	Mostly stable	Highly unstable	Bank undercut	Yes/No		
	Bank cover	Soil ☐	Stony ☐	Grass ☐	Tussock ☐	Shrubs ☐	Trees ☐	Artificial ☑
In-stream	Stream bed substrate	Clay/mud ☐	Silt/sand ☑	Gravel ☑	Cobble ☐	Boulder ☐	Bedrock ☐	Artificial ☐
	Bed stability	Highly stable	Moderately stable	Highly unstable ✓			* In-stream notes	
	Macrophytes	Submerged ☐	Marginal ☐	Emergent ☑				
	Periphyton	None visible	Sparse ✓	Common	Abundant	Dominating		
	Wood	Absent ✓	Sparse	Common	Abundant	Dominating		
	Moss	Absent ✓	Sparse	Common	Abundant	Dominating		
	Leaves	Absent	Sparse	Common ✓	Abundant	Dominating		
	Shading	Open ✓	Partial	Heavily shaded	Overhanging vegetation	Yes/No		
Riparian & Catchment	Riparian width	L – 1 m	R – 2-3 m	Stock access	L – Yes/No	R – Yes/No	* Riparian & catchment notes	
	Stock damage	None	Minor	Moderate	High			
	Problem plants	Yes/No	Photo taken – Yes/No		Type(s)			
	Riparian cover	Soil ☑	Rock/ gravel ☐	Grass ☐	Tussock ☐	Wetland plants ☐		
		Ferns ☐	Shrubs ☐	Native trees ☐	Deciduous exotic ☐	Conifers ☐	Other ☐	
	Adjacent land use	Conservation/ reserve ☐	Short grazed ☐	Long ungrazed ☐	Production forest ☐	Dairy cattle ☐	Beef cattle ☐	Sheep ☐
		Crop ☐	Horticulture ☐	Deer ☐	Horse ☐	Urban ☑	Road ☐	Other ☐
	Catchment land use	Native forest ☐	Plantation forest ☐	Farming ☐	Urban ☑	Industry ☐	Mining ☐	Other ☐