ACCUMULATION OF TRACE ELEMENTS IN AQUATIC FOOD CHAINS DUE TO SEA-FILL ACTIVITIES

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ABSTRACT

Elevated levels of trace elements in the environment are of great concern because of their persistence, and their high potential to harm living organisms. The exposure of aquatic biota to trace elements can lead to bioaccumulation, and toxicity can result. Furthermore, the transfer of these elements through food chains can result in exposure to human consumers. Sea-fill or coastal fill sites are among the major anthropogenic sources of trace elements to the surrounding marine environment. For example, in the Maldives, Thilafushi Island is a sea-fill site consisting of assorted municipal solid waste, with multiple potential sources of trace elements. However, there is limited data on environmental trace element levels in the Maldives, and although seafood is harvested from close to this site, there is no existing data regarding trace element levels in Maldivian diets. Following the Christchurch earthquakes of 2011, "clean" rubble was used to create a sea-fill in Lyttelton Harbour, potentially altering the environmental trace element profile in this setting, and again creating a potential threat in terms of toxicity to both marine biota and human consumers of seafood from this site. This thesis sought to investigate the sea-fill contributions to trace element contamination in these two distinct sea-fill sites, and to determine the impact of this in an aquatic food chain, including possible risks to human consumers.

In order to accurately determine trace element levels from natural seawater, a study was conducted to validate the use of iminodiacetate resin as a solid phase extraction method. Once the utility of this technique was verified, a baseline study of trace elements in seawater, sediment and three species of shellfish around the wider

Lyttelton Harbour was performed. This study showed that the level of site contamination was reflected in the trace element body burdens of green-lipped mussel, cockle, and pipi. The calculated biota sediment accumulation factor (BSAF) values suggested that cadmium was the trace element most efficiently transferred from sediment to organism. Data also indicated that, in general, green-lipped mussels accumulated greater levels of trace elements than cockles and pipi, with the exception of arsenic and copper which were most elevated in cockles.

This baseline study was then extended to investigate the trophic transfer of trace elements in a Lyttelton Harbour food chain. These data indicated that mercury was the only element that showed any appreciable level of biomagnification through the food chain. The results of this study suggested that dietary uptake of trace elements is an important route for bioaccumulation in animals, and that in general, body burdens do reflect environmental trace element levels. This investigation also established for the first time, baseline levels of trace elements in key Lyttelton Harbour food chain species such as crabs, and fish (banded wrasse and spotty).

Investigation of trace element contamination of seawater, marine sediments, and marine biota were then conducted at the sea-fill sites of Thilafushi Island and Lyttelton Harbour. Significantly higher concentrations of trace elements, and higher metal pollution index (MPI) values were measured at the two sea-fill sites relative to their reference sites. At Thilafushi Island, regulatory limits for both copper (seawater; 80% protection limit; sediment ISQG-low) and zinc (sediment ISQG-high) were exceeded. Similarly, copper concentrations in seawater exceeded the 90% protection level and sediment lead exceeded the ISQG-low value at the sea-fill site of Lyttelton Harbour. In marine biota, lead levels in red mullet and cadmium in penguin wing oysters collected from the Thilafushi sea-fill site exceeded the food standard maximum allowable levels (ML) for fish and molluscs, respectively. Conversely, no biota sample from the sea-fill of Lyttelton Harbour exceeded ML values.

Risk assessment for consumption of seafood from the Thilafushi sea-fill showed that the estimated weekly intake values for inorganic arsenic, lead and mercury can exceed the provisional tolerable weekly intake (PTWI) value for all population categories (toddler, child, male and female adult) in the Maldives. This indicates potential risks for consumption of seafood from the vicinity of the Thilafushi

sea-fill site. Moreover, the high fish consumption rate in the Maldives can result in exceedance of PTWI values even through the consumption of seafood with concentrations lower than the ML values. The risk assessment for consumption of sea food from Lyttelton Harbour sea-fill showed little risk, with only shellfish consumption at high levels exceeding PTWI values.

List of Acronyms

AAS	Atomic Absorption Spectrometer
AFPs	Animal Fibrous Proteins
ANOVA	Analysis of Variance
ANZECC	Australia and New Zealand Environment and Conservation Council
BSAF	Biota Sediment Accumulation Factor
BW	Body Weight
CCC	Christchurch City Council
CGIAR	Consultative Group on International Agricultural Research
CI	Confidence Interval
EC	European Commission
ECan	Environment Canterbury (Canterbury Regional Council)
EIA	Environment Impact Assessment
EVISA	European Virtual Institute for Speciation Analysis
EWI	Estimated Weekly Intake
FAAS	Flame Atomic Absorption Spectrometry
FAO	Food and Agricultural Organization
FDEP	Florida Department of Environmental Protection
FSANZ	Food Standards Australia New Zealand
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ISQG	Interim Sediment Quality Guideline

JECFA	Joint FAO/WHO Expert Committee on Food Additives
KED	Kinetic Energy Discrimination
LH	Lyttelton Harbour
LOD	Limit of Detection
LOQ	Limit of Quantification
LPC	Lyttelton Port of Christchurch
MFDA	Maldives Food and Drug Authority
ML	Maximum Allowable Level
MPI	Metal Pollution Index
MRG	Metal Rich Granules
MSW	Municipal Solid Waste
MTLP	Metallothionein-like Proteins
NIST	National Institute of Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
NRCC	National Research Council Canada
NZTDS	New Zealand Total Diet Study
ORS	Octopole Reaction System
PC2	Physical Containment Level 2
PG	Pigeon Bay
PTWI	Provisional Tolerable Weekly Intake
QA	Quality Assurance
QC	Quality Control
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
SPR-IDA	Suspended Particulate Reagent Iminodiacetate
SRM	Standard Reference Material
STS	Shield Torch System
TOC	Total Organic Carbon
TTP	Trophic Transfer Potential
USEPA	US Environmental Protection Agency
USFDA	US Food and Drug Administration
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Trace element pollution in the marine environment has become a global concern due to the potential for harmful impacts on marine ecosystems and human health. Advances in industrialisation, pressures of population increases such as elevated municipal solid waste (MSW) generation, and increased commercial and agricultural operations inevitably increase the release of potentially toxic chemicals into the environment (Wong et al. 2006). In recent years the utilisation of coastal landfills and sea-fills, has been increasing, but the impacts of these waste management approaches are not well understood. Of particular concern is the release of trace elements into the surrounding marine environment from these fills (Ettler et al. 2008; Ettler et al. 2006; Jones 2010; Kersten et al. 1997). Trace elements can bioaccumulate and transfer within aquatic food chains, causing a number of effects within the nearby marine ecosystem, but also potential impacts on human consumers (Adriano 2001; Oronsaye et al. 2010; Rodriguez & Reynoldson 2011).

One example of human impact following contamination of the marine environment is that of Minamata disease in Japan in the 1950's. In this scenario mercury contamination in the marine environment from industrial waste discharge to Minamata Bay lead to the accumulation of methylmercury in seafood that was then consumed by local populations. This resulted in severe direct and indirect neurological impacts on the offspring of mothers who consumed the seafood during pregnancy (Kessler 2013). This incident raised significant concern about the transfer

of toxic elements in marine food chains to humans (Gochfeld 2003; Wang & Rainbow 2008). Long term exposure to high levels of trace elements can cause severe health conditions including behavioural changes, permanent damage to vital organs, life threatening chronic diseases such as cancer, and may also lead to death (D'Souza et al. 2003; Hartwig et al. 2002).

Although there has been an extensive amount of work monitoring trace elements in the environment around the world, including New Zealand and Australia (Acosta et al. 2010; Fabris et al. 2006; Jones 2010; Meador et al. 2004; Peake et al. 2006; Usero et al. 2005; Williamson et al. 1996), few studies have been undertaken in the developing world, where most of the trace element contamination is occurring (Li et al. 2009; Pacyna & Pacyna 2001). This lack of knowledge extends to the Maldives, a small island nation in the Indian Ocean, with significant waste management issues. One solution for MSW is the use of a sea-fill, in the form of a converted lagoon. The environmental consequences of this strategy, with respect to trace element contamination are unknown. There is even a lack of baseline data regarding trace elements, be it in the environment of the Maldives or the human food chain (CDE Consulting 2011). Consequently, the two goals of this thesis were to address this data gap, and perform a survey of trace element contamination related to sea-fill activities, and to determine the potential risk of this contamination to consumers of potentially-contaminated seafood.

Knowledge of trace element contamination in the developed world, including New Zealand, is much better than in countries such as the Maldives (Glasby et al. 1988; Kennedy 1986; Redfern 2006; Williamson et al. 1996). However, for sites such as Lyttelton Harbour, the main port serving the city of Christchurch, there is relatively limited information regarding trace element concentrations and their potential risk to the marine ecosystem and seafood consumers (Sneddon & Barter 2009). Furthermore, in recent years, a sea-fill has been added to the inner harbour, comprising of “clean” rubble sourced from buildings damaged in the 2011 Canterbury earthquakes. This could be a potential source of trace elements to the marine ecosystem, and could also impact human trace element intake through the consumption of seafood from the harbour. The second goal of the current thesis was to investigate the trace element profile of Lyttelton Harbour and to assess the potential impact of this sea-fill site on

environmental and human health.

1.1 TRACE ELEMENT CONTAMINATION IN THE MARINE ENVIRONMENT

The term “trace element” refers to a group of metals and metalloids that occur naturally in the ecosystem at low concentrations (Adriano 2001; Appenroth 2010; O'Neill 1998), and includes arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), mercury (Hg), lead (Pb) and zinc (Zn). Industrialisation and urbanisation increase the anthropogenic contribution of trace elements to the environment (Crain et al. 2009), and as elements do not biodegrade (Crnkovic et al. 2006; O'Neill 1998; Wong et al. 2006), they consequently circulate in biogeochemical cycles within the environment (Adriano 2001; O'Neill 1998). Trace elements are of great concern because of their high potential to harm living organisms in small amounts. Non-essential trace elements (e.g. arsenic, cadmium, mercury and lead) have no known biological functions and cause health issues in organisms at trace concentrations (Guérin et al. 2011; Rainbow 1993,2007). Copper, iron and zinc are essential trace elements that are required in small amounts to support physiological processes (Mertz 1981). However, at higher concentrations these essential trace elements can be toxic (Adriano 2001; Mertz 1981; Rainbow et al. 2004).

Natural sources of trace elements to the marine environment include weathering of soil and rocks, and volcanic discharges (Ahlf et al. 2009; Akter et al. 2005; Ochieng et al. 2007,2009). Anthropogenic sources of trace elements into the marine environment include industrial wastes, agricultural runoff, municipal solid wastes in coastal landfills or sea-fills, mining activities, smelting, dredging activities, boating activities (e.g. anti-fouling paints), geothermal discharges, domestic effluents and storm water runoff (Abrahim & Parker 2002; Acosta et al. 2010; Akter et al. 2005; Crnkovic et al. 2006; Fakayode & Olu-Owolabi 2003; Williamson et al. 2003).

Marine sediments act as a sink for contaminants received by the environment (Harbison 1986; Williamson et al. 1996). Trace elements released into the aquatic environment are preferentially deposited in the benthic sediments of coastal waters, in

particular onto the fine particles (Denton & Morrison 2009; Jones 2010; Maata & Singh 2008; Naidu & Morrison 1994; Williamson et al. 1996), from which they can be released to the environment by various processes of remobilisation (Saulnier & Mucci 2000). Trace elements are often adsorbed on clay, organic matter, oxides and hydroxides of iron (reducible-Fe^{3+} or oxidisable-Fe^{2+} forms), and manganese, calcium carbonates (Agah et al. 2009; Elderfield et al. 1981; Smedley & Kinniburgh 2002), as well as other exchangeable and more strongly bound forms (Ibhadon et al. 2004; Smedley & Kinniburgh 2002). When not bound in sediments, trace elements may exist in the dissolved phase as metal ions (M^{2+}), dissolved inorganic metal–ion pairs ($M–OH^{+}$, $M–Cl^{+}$, $M–CO_3$, M-S), organic forms (M–DOM; dissolved organic matter) and as colloidal forms in both porewater and the water column (Smedley & Kinniburgh 2002; Vink 2009).

Trace elements that are available for organisms to take up from the environment are termed bioavailable (Luoma & Rainbow 2005; Rainbow 2007). The increase in concentrations of trace elements in a biological organism is called bioaccumulation, and the increase in the concentrations of trace elements in a biological organism through trophic levels is called biomagnification (Adriano 2001; Barwick & Maher 2003; Nfon et al. 2009; Rainbow 2007). The bioavailability, and hence bioaccumulation, of an element depends on various geochemical and biological factors (Al-Weher 2008; Boening 1999; Rainbow 2007; Rodriguez & Reynoldson 2011; Vink 2009).

Chemical speciation plays a vital role in the bioavailability of trace elements (Adriano 2001; Ahlf et al. 2009; Chakraborty & Owens 2014). Chemical speciation refers to the different forms (e.g., different oxidation states, complexes with other chemicals) of trace elements that exist in an aquatic system. It is often determined by factors such as pH, temperature, ionic strength, dissolved oxygen, solubility, and the availability of other chemicals and ligands (Boening 1999). For example, the most bioavailable form of lead in the aquatic environment is soluble Pb^{2+} ion, and it is mainly present in the form of lead carbonate ($PbCO_3$) and lead hydroxide ($Pb(OH)_2$) (Ibhadon et al. 2004). As another example, it is believed that iron oxide-bound cadmium is more bioavailable than organic particulate bound cadmium (Ahsanullah et al. 1984). Water chemistry largely dictates availability of trace element to uptake from

the dissolved phase, but for most trace elements the major source to an organism is the diet (Fauchald & Jumars 1979; Maher 1985; Reinfelder et al. 1998). Consequently, trace elements bound to organic ligands such as amino acids may become more bioavailable, and factors such as digestive tract pH, and the physiology of the intestine play important roles in dietary accumulation, and consequently the passage of metals through the food chain (Rainbow et al. 2006a).

1.2 TRACE ELEMENTS: PROPERTIES, USES AND SOURCES OF CONTAMINATION TO THE MARINE ENVIRONMENT

The main focus of this thesis was to investigate four toxic trace elements: arsenic, cadmium, mercury and lead. However, nutrient trace elements such as copper, iron and zinc, are also prominent in aquatic settings and can also produce toxic effects at higher concentrations. Therefore, these essential elements were also investigated in this study along with the non-essential elements.

1.2.1 ARSENIC

Although arsenic is in group five, and is thus classified as a non-metal in the periodic table, it is often referred to as a metalloid, because it shares similar physical and chemical properties with both metals and non-metals (Akter et al. 2005; Rainbow et al. 2006a). Arsenic can be found in the natural environment in both organic and inorganic forms, and in several oxidation states (-3, 0, +3 and +5) (Akter et al. 2005; Eisler 1988; Smedley & Kinniburgh 2002). In natural waters it is mostly found as inorganic arsenite [As^{3+}] or pentavalent arsenate [As^{5+}] (Smedley & Kinniburgh 2002). As^{3+} is more toxic than As^{5+}, which in turn is more toxic than methylated or organic arsenic species (Sharma & Sohn 2009; Smedley & Kinniburgh 2002; WHO 2001). It is the less toxic organic arsenic species such as arsenobetaine and arsenosugars that are the predominant forms found in marine organisms (Francesconi & Edmonds 1998; Neff 1997).

Arsenic is used in pesticides, wood preservatives, semi-conductors, glass and

enamels (Denton et al. 1997). The anthropogenic sources of arsenic to the marine environment include agricultural runoff from use of fertilisers and pesticides, urban runoff, industrial waste, treated woods and timbers, and municipal solid wastes. Toxicity associated with arsenic exposure can lead to a range of diseases such as skin disorders, lung diseases, liver diseases, peripheral vascular disease, hypertension, heart disease, and cancer of skin, lung and urinary bladder (Fatmi et al. 2009; Graeme & Pollack 1998; Mazumder 2008).

1.2.2 CADMIUM

Although cadmium and zinc are placed in the same group in the periodic table, cadmium is considered a non-essential element while zinc is classified as an essential element. Cadmium is able to displace zinc from zinc-containing enzymes (O'Neill 1998) and can therefore inhibit the essential roles of these enzymes in the body. Although the chemical properties of cadmium and zinc are very similar, the hydrated zinc ion (Zn^{2+}) is relatively more stable than the hydrated form of cadmium ion (Cd^{2+}) in aqueous form (O'Neill 1998). Cadmium is able to compete (i.e. bind) more strongly than zinc for binding sites, and thus is favoured in terms of formation of metal-sulphur bonds (O'Neill 1998).

The general uses of cadmium include electroplating, plastic stabilisers, pigments, plastics, glass, ceramics, semiconductors, and in nickel-cadmium batteries (Hutton 1983; O'Neill 1998). The major anthropogenic sources of cadmium to the environment are the steel industry, waste incineration, mining activities, agricultural runoff from use of phosphate fertilisers, and zinc production (GESAMP 1985; Hutton 1983). The toxic effects of cadmium include kidney failure, itai-itai disease, disruption of enzymatic pathways, anaemia, liver disorders, weakening of bones that can lead to osteoporosis as seen in itai-itai disease (which also requires a low calcium intake), and lung cancers (Finkelman 2005; GESAMP 1985).

1.2.3 ZINC

The major uses of zinc include zinc-based alloys, brass and bronze, galvanising works, paints, manufacturing of batteries and rubber materials as well as in sacrificial anodes on marine water craft (Denton et al. 1997). The sources of zinc to the marine environment include storm water discharge, burning of fossil fuels, municipal solid

wastes, brass and galvanised fittings on boats, zinc-based anti-corrosion and anti-fouling paints on boats, and from applications of fertiliser and pesticides near coastal areas (Denton et al. 1997). Although zinc is an essential element, excess intake can disrupt essential enzymatic functions in both humans and aquatic organisms (Berthet et al. 2003; O'Neill 1998; Rainbow 2007).

1.2.4 LEAD

Lead is one of the few trace elements that can be found in its metallic form in nature, but more frequently lead is present in its +2 oxidation state (O'Neill 1998). Organolead compounds, particularly alkyl-lead forms, are considered more toxic than other species of lead (O'Neill 1998). Lead in the marine environment can precipitate as lead sulphide, an insoluble compound, and often exists as forms with low bioavailability such as bound to suspended sediment particulate matter (O'Neill 1998). These forms of lead can be remobilised by changing environmental conditions, such as a reduction in pH (Kjeldsen et al. 2002). Approximately 5% of the lead in aquatic systems is in the dissolved form (O'Neill 1998). Like most trace elements, lead has a high affinity for thiol (-SH) groups in biological molecules and can strongly bind proteins, and also nucleic acids (O'Neill 1998).

The key uses of lead are in lead-acid storage batteries in motor vehicles, lead alkyl compounds that are added to petrol to reduce knock in combustion engines, as electrodes in electrolysis, in pigments, lead paints, solder, anti-fouling paints and as stabilisers in plastics (Chen et al. 2005; Denton et al. 1997; Järup 2003; O'Neill 1998; Wu et al. 2000). The anthropogenic sources of lead to the marine environment include discharge from manufacturing processes such as metal processing works, discharge from mining activities, combustion of leaded fuels, burning of wood and coals, solid waste incineration, atmospheric deposition, domestic wastewater and sewage wastes (Cabral-Oliveira et al. 2015; GESAMP 1985). Lead toxicity can manifest as a wide range of impacts such as impairment of blood synthesis, hypertension, hyperactivity, bone defects, weakness in fingers, wrists and ankles, miscarriage in pregnant woman, and brain damage (D'Souza et al. 2003; Finkelman 2005; GESAMP 1985; O'Neill 1998).

1.2.5 MERCURY

Mercury is the only metal that is liquid at room temperature, and it is very volatile in air. Most compounds of mercury are also volatile (Gochfeld 2003; O'Neill 1998). Mercury exists in the 0, +1, +2 oxidation states and methylation is a very important feature of mercury cycling in the aquatic environment. The main form of mercury found in fish is methylmercury, which generally accounts for over 90% of total mercury (Hight & Cheng 2006; Olmedo et al. 2013). Mercury entering aquatic systems will be methylated by microorganisms and abiotic processes and converted to methylmercury, a form that has high bioavailability, and which biomagnifies through food chains (Gochfeld 2003; O'Neill 1998). This is also a form of mercury that is higher in toxicity than inorganic species (Gochfeld 2003; Monperrus et al. 2007). Under alkaline conditions, sulphide ions (S^{2-}) can turn soluble mercury compounds into insoluble mercury (II) sulphide, and when the pH of the system decreases, it favours solubilisation, and hence greater potential for the synthesis of methylmercury by microorganisms (Gochfeld 2003; O'Neill 1998).

Mercury is used in the production of chlorine and acetaldehyde, electrical equipment, instruments such as thermometers, as a catalyst in the production of plastics, in pesticides, as a preservative in vaccines, in pharmaceuticals, in the dental industry and as a component of anti-fouling paints (Denton et al. 1997; Fimreite 1970; Gochfeld 2003). Sources of mercury to the marine environment include coastal or sea-fill activities, industrial waste, discharge from mining activities, sewage outfalls and atmospheric deposition (Heck et al. 1994; Wuana & Okieimen 2011). Mercury toxicity includes severe kidney damage, neurological disruption, and behavioural disturbances (Langford & Ferner 1999).

1.2.6 COPPER

Copper is an essential trace elements that is moderately abundant in the natural environment. Copper presents in the aquatic environment primarily in the +2 oxidation state (e.g. $CuOH^{+}$, $CuCO_3$, $CuSO_4$), but also in the cuprous form (Cu^{+} which rapidly becomes Cu^{2+} in the aquatic environment) (Callender 2003). Copper is known to have high affinity for clay mineral fractions, especially organic carbon and manganese oxides, and concentrations of copper in the aquatic environment are

strongly dependent on the type and concentration of inorganic and organic ligands present (Callender 2003). In this regard, numerous studies have demonstrated that aquatic copper toxicity can vary depending on the type of complexing ligand and the concentration of cations that may compete with copper for binding sites and uptake pathways (McGeer et al. 2002; Paquin et al. 2002; Santore et al. 2001).

Copper is used in the electrical industry, as a catalyst in alloy form, as a wood preservatives, in pesticides, and as a component of anti-fouling paints (Denton et al. 1997; Santore et al. 2001). Sources of copper to the marine environment include discharge from mining and smelting activities, domestic and industrial wastewaters, steam electrical production, MSW disposal at coastal sites, incineration emissions, sewage outfalls, antifouling paints, wood preservatives, port operation activities, and urban stormwater runoff (Denton et al. 1997; Jones 2010; O'Neill 1998; Williamson et al. 2003). Although copper is an essential element in several enzymes and is involved in the synthesis of haemoglobin, excess exposure to copper can result in various toxic effects in humans including irritations in nose, mouth, and eyes, severe headaches, dizziness, nausea, and diarrhoea (Finkelman 2005).

1.2.7 IRON

Iron is an essential element found in various oxide forms, and often presents in the environment as divalent iron (Fe^{2+}) and trivalent iron (Fe^{3+}) compounds. Iron is not often considered a toxic trace element in the environment, and it is usually found at high levels in both marine sediments (Jones 2010; Turner 2000), and biota (Brooks & Rumsey 1974; Kennedy 1986; Turoczy et al. 2001). Uses of iron are numerous, and include utility as a construction material in ships, heavy vehicles and buildings, and as piling materials in wharfs and seawalls. Anthropogenic sources of iron to the marine environment include industrial discharge, stormwater runoff, acid mine drainage, port operation works, ship hulls, wharves, and coastal fill sites (ECan 2008; Jones 2010; Winterbourn et al. 2000).

1.3 SOLID WASTE DISPOSAL AND SEA-FILL

Increased human activities throughout the world significantly increase the

volume of solid waste generated (Turan et al. 2009; Wong et al. 2006). The common methods of solid waste disposal are open site dumping, landfills and incineration (Kinnaman 2009; Turan et al. 2009). The most economical and common method of solid waste disposal in many countries is either burial in landfills, or incorporation into sea-fills (Khoury et al. 2000; Kjeldsen et al. 2002). Studies on coastal fill and sea-fill activities have shown that high levels of trace elements can be released into the surrounding marine environment from the fill materials (Chifamba 2007; Jones 2010). Therefore, improper management of solid waste can cause serious environmental and health consequences, due to the risks associated with leaching of contaminants, including trace elements from waste disposal sites (Christensen et al. 1994; Jones 2010; Kjeldsen et al. 2002).

1.3.1 SOURCES OF TRACE ELEMENTS IN FILL MATERIALS

Sources of trace elements in MSW include batteries, consumer electronics, ceramics, light bulbs, house dust and paint chips, lead foils, used motor oils, plastics, inks and glass (Whittle & Dyson 2002). Sources of arsenic in MSW are wood preservatives, paints, dyes, ceramics, glass, electronics, pigments, and antifouling agents (Akter et al. 2005; Leonard 1991). The primary source of cadmium in MSW is rechargeable nickel-cadmium batteries and some plastic materials, while lead comes from variety of sources like plastics, road dust and paint chips (Heck et al. 1994). The main contributors of mercury to MSW are used household batteries, broken thermometers, and fluorescent lamps (Heck et al. 1994). Sources of copper, iron and zinc in MSW include electric wiring materials, galvanised materials, scrap metals, cooking pots, kitchen wares, plastic materials, treated woods and cardboards, paper material, and food tins and containers (Chifamba 2007; Long et al. 2011).

Sources of trace elements in fill sites considered to contain clean materials (i.e. building materials), such as the one in Lyttelton Harbour, include treated timbers, concrete reinforced metal bars, paint chips, plastics, electrical ducting, elemental copper in the form of cabling, cable sheathing and panel products (LPC 2011; Sneddon 2011). These materials may be inadvertently incorporated in fill materials, and are known to release trace elements to the surrounding environment (Akter et al. 2005; Denton et al. 1997; Heck et al. 1994; Leonard 1991).

1.3.2 LEACHATE GENERATION FROM FILL ACTIVITIES

In landfills, leachate is generated as rainwater passes through the layers of the landfill, acting to transfer the pollutants into the percolating water (Ettler et al. 2008; Kjeldsen et al. 2002). Marine landfills or sea-fills are subjected to similar processes via seawater percolation (Kjeldsen et al. 2002). When seawater levels rise and fall with the tide, seawater penetrates through the layers of wastes creating leachate (Jones 2010). Studies on leaching from fill activities near coastal sites and sea-fill have reported that contaminant levels in seawater around the dump site were higher than the levels found in waters more distant from the waste site (Jones 2010; Maata & Singh 2008; Naidu & Morrison 1994).

Solid wastes in fill activities undergo physical, chemical and biological degradation processes as the refuse decomposes (Kjeldsen et al. 2002; Taulis 2005). The concentration and composition of leachates may differ depending on the waste type in the fill, and the decomposing environmental conditions (i.e. aerobic or anaerobic) (Kjeldsen et al. 2002). There are four distinctive stages of refuse decomposition in terrestrial landfills. These are the aerobic phase, the anaerobic acid phase, the initial methanogenic phase, and the stable methanogenic phase (Farquhar & Rovers 1973; Kjeldsen et al. 2002). When the decomposing condition is aerobic, a number of biological and chemical reactions can occur leading to the anaerobic acid phase, where lowering of pH occurs, increasing the oxidation-reduction potential, and increasing the cation exchange capacity of the refuse (Kjeldsen et al. 2002). The increase in oxidation-reduction potential can increase the formation of oxidised functional groups such as carboxylic acids on humic matter (Kjeldsen et al. 2002). These alterations in the refuse decomposition process can result in an overall increase in trace element mobilisation, and increased concentrations of trace elements in the leachate (Khoury et al. 2000; Kjeldsen et al. 2002). Little work appears to have been carried out to establish whether the characteristics of terrestrial fills are also present in marine fills.

Leachates contain a complex mixture of contaminants, and the broader categories of contaminants in leachate include dissolved organic matter, inorganic macrocomponents, trace elements and xenobiotic organic compounds (Christensen et al. 1994; Jones 2010; Kjeldsen et al. 2002). Dissolved organic matter includes

components such as volatile fatty acids and fulvic-like and humic-like compounds. Inorganic macrocomponents include calcium, magnesium, sodium, potassium, ammonium, chloride, sulphates, and hydrogen carbonates. Xenobiotic organic compounds include a variety of aromatic hydrocarbons, phenols, chlorinated aliphatics, pesticides, and plasticisers. Trace elements present in leachates include arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel and zinc (Kjeldsen et al. 2002). A wide variation in trace element concentrations has been reported for different landfill sites (Table 1.1) (Kjeldsen et al. 2002).

Table 1.1: Range of trace element concentrations in various landfill leachates

Trace element	Concentration in landfill leachate (µg L^{-1})
Arsenic	10 - 1000
Cadmium	0.1 - 400
Copper	5 - 10000
Iron	3000 - 5500000
Mercury	0.05 - 160
Lead	1 - 5000
Zinc	30 - 1000000

Adapted from Kjeldsen et al. (2002)

Trace elements deposited in fill activities can be released from the fill materials, a process that may take decades (Flyhammar 1995; Kjeldsen et al. 2002). However, there are processes that will also act to slow leaching. For example, Belevi & Baccini (1989) and Bozkurt et al. (2000) predicted that trace elements can be immobilised for hundreds of years by the alkaline conditions generated by the stable phase of decomposition. Sorption to organic matter and soil, and precipitation are also thought to play a significant role in immobilising trace elements in refuse (Kjeldsen et al. 2002). In addition, sulphides (formed by reduction of sulphates under the alkaline conditions generated at the stable methanogenic phase) can immobilise trace elements by formation of insoluble metal sulphides, which explains the low concentration of trace elements measured in some leachates (Christensen et al. 1994).

1.4 TRACE ELEMENT ACCUMULATION IN AQUATIC FOOD CHAINS

Contaminants, including trace elements, leaching from fill activities near coastal areas can enter into the coastal zone (Denton & Morrison 2009; Jones 2010; Maata &

Singh 2008), and become bioavailable for uptake by aquatic organisms. Microorganisms such as phytoplankton and zooplankton take up these trace elements from the surrounding water (Chen et al. 2000; Mason et al. 1996). Subsequently, the consumption of these organisms results in the passage of the trace elements through the food chain (Ahlf et al. 2009; Chen et al. 2000; Falconer et al. 1983; Mason et al. 1996). Some of these trace elements will be bioaccumulated, and some (e.g. mercury) will biomagnify as they pass to higher trophic levels (Blackmore & Wang 2004; Reinfelder et al. 1998; Wang 2002). However, diet is not the only pathway for trace element uptake by marine organisms. Some animals can absorb dissolved trace elements via gills and/or skin, while other organisms can take up trace elements from the particulate phase (Ahlf et al. 2009; Marsden et al. 2014; Wang & Rainbow 2008).

1.4.1 TOXICITY OF TRACE ELEMENTS TO MARINE ORGANISMS

Trace elements bioaccumulate in marine biota, and can produce harmful impacts on aquatic organisms at higher exposure levels (Filipovic Marijic & Raspor 2007). Trace element contamination in the aquatic environment can affect the quality of water and can result in bioaccumulation of these elements in aquatic life, with potential long-term implications for ecosystem health (Ip et al. 2007). Accumulation of trace elements may impact physiological function and disrupt growth and reproduction (Bowmer et al. 1994; Chandurvelan et al. 2012). At a molecular, biochemical and cellular level the mechanisms of trace element toxicity in aquatic organisms are similar to those of humans (see Section 1.4.2). In aquatic biota these cellular events result in ecologically-important effects such as the impairment of growth, development and survival. For example, cadmium, zinc and lead can cause injury to fish gills and kidneys, and can cause excess mucus production followed by failure of respiratory function (Andreji et al. 2006). In shellfish, cadmium can decrease feeding rates, and impair cellular, biochemical and physiological systems (Chandurvelan et al. 2012). Toxicity studies indicate that exposure of marine phytoplankton to mercury can cause significant inhibition of growth, while significantly reducing the rate of photosynthesis (Wu & Wang 2011). Likewise, trace element exposure studies on the American oyster (*Crassostrea virginica*) indicate that mercury, copper and zinc can significantly inhibit the development of oyster embryos (Calabrese et al. 1973).

As in humans, the toxic effects of trace elements are more prominent in juvenile stages (Grosell et al. 2007). For example, copper has been shown to be significantly more toxic in juvenile fish, and larval polychaete worms and crustaceans, than in adults of the tested species (Grosell et al. 2007; Brown and Ahsanullah 1971). At least in the case of fish, this is because of the higher demand of juvenile fish for sodium uptake, a process inhibited by copper (Grosell et al. 2007).

The toxicity of trace elements can also vary depending on the species of marine organism (Graeme & Pollack 1998; Rainbow 2007). For example, marine worms have been shown as more susceptible to the toxic effects of lead, copper and zinc than shrimp (Brown & Ahsanullah 1971). Demonstrating that these effects are also trace element-dependent, both shrimp and annelid species were equally susceptible to the impacts of mercury. Speciation of the trace element also plays a role in toxicity. For example inorganic mercury was found to produce less toxic effects than methylmercury in phytoplankton (Wu & Wang 2011).

1.4.2 TOXICITY OF TRACE ELEMENTS TO HUMANS

Humans can be exposed to trace elements via food, water, and to a much lesser degree, via the air and through dermal contact (Akter et al. 2005; O'Neill 1998). Among the trace elements, lead, mercury, cadmium and arsenic are of great concern (O'Neill 1998), primarily due to their high potential to accumulate in the food chain and cause harmful effects on organisms (Ikemoto et al. 2008). Once these toxic trace elements are present in the body, they can mimic essential elements such as iron or calcium and displace them from their normal binding sites to produce some of their biochemical activities (Ahlf et al. 2009; Hartwig et al. 2002). Displacement of metal co-factors of enzymes can inhibit enzyme function, and in turn inhibit critical cellular activities (Akter et al. 2005; Wang & Rainbow 2010). Toxicity may also be mediated by the binding of these elements with sulphydryl (SH) and hydroxyl (OH) groups of amino acids or proteins, haemoglobin, RNA and DNA. A third mechanism of toxicity that may relate to the first two is the generation of oxidative stress (O'Neill 1998; Wang & Rainbow 2010). Oxidative stress occurs through the generation of reactive oxygen species, formed via the Fenton reaction. The displacement of iron from this reaction by other trace elements can trigger enhanced reactive oxygen species production, while trace elements may also bind to thiol groups and decrease activity

of important oxidative defence mechanisms such as the intracellular reactive oxygen scavenger, glutathione (Ercal et al. 2001; Valko et al. 2005). Oxidative stress can manifest as DNA adducts, the formation of protein carbonyls and lipid peroxidation, all of which can cause significant toxicity. At a whole organism level, toxicity resulting from trace element exposure can manifest in a range of symptoms, including alteration of cognitive behaviours, vision and hearing impairment, damage to vital organs such as kidney and brain, impaired bone metabolism, and both acute and chronic illnesses (Bergomi et al. 2005; Graeme & Pollack 1998; Rose et al. 1992; Sulkowski et al. 2000; Wu et al. 2000).

Although essential trace elements (e.g. copper, iron, zinc) are required for physiological functions, they can become toxic at elevated concentrations (Berthet et al. 2003; Rainbow 1985; Rainbow et al. 2006a). For example, excess intake of copper can result in the accumulation of this element in brain, liver, pancreas, and the myocardium, causing detrimental effects (Tuzen et al. 2005). Similarly, although zinc is an essential element, vital for the functioning of enzymes, excess zinc can disrupt a number of processes in both humans and aquatic organisms (Berthet et al. 2003; O'Neill 1998; Rainbow 2007).

In general, excessive levels of trace elements have their most significant impacts on the developing child (Kaye 2004; O'Neill 1998). Prenatal exposure to trace elements such as mercury and lead can cause neuropsychological developmental defects, alterations in sexual and functional development and other foetal abnormalities (Bjerregaard & Hansen 2000; Butler Walker et al. 2006). Young children and infants are very vulnerable to the effects of trace elements because of their higher metabolic rates, in combination with more efficient absorption and retention of these elements, and reduced defences against their toxic effects (D'Souza et al. 2003; Godwin 2001). For example, children absorb a greater proportion of ingested lead than adults, and the ingested lead is distributed differently (Godwin 2001; O'Neill 1998). Children accumulate about 28% of lead in their soft tissues including the brain, whereas only 5% accumulates in adult soft tissues (O'Neill 1998).

Wide-scale human poisoning by trace elements in food occurred in the 1950's in Japan, where the local villagers consumed fish and shellfish that had bioaccumulated mercury from Minamata Bay. This mercury poisoning led to

Minamata disease, and killed hundreds of people, while several thousand people were left paralysed due to neurological impairment (Kudo et al. 1998). In 1972, consumption of seed grains treated with methylmercury fungicide in Iraq killed an estimated 10,000 people, and over 100,000 people were severely and permanently brain damaged (Gochfeld 2003; Li et al. 2009). Similarly, cadmium in food has also caused human toxicity. Prolonged consumption of rice harvested around a cadmium contaminated area caused "itai–itai" (ouch ouch) disease, in the Toyama Prefecture of Japan in 1912. This often fatal disease caused severe injuries to kidney and bones (Inaba et al. 2005; O'Neill 1998).

Intake of drinking water with elevated arsenic is considered the main pathway of arsenic exposure in humans (Akter et al. 2005; Das et al. 2004; Smedley & Kinniburgh 2002). Previous studies showed arsenic concentrations exceeded food safety limits in rice grown in arsenic-contaminated sites (Das et al. 2004; Meharg & Rahman 2002). A dietary study in Cambodia showed that fish was the foodstuff that contained the highest concentrations of total arsenic, and highlighted that fish consumption was also the main route of arsenic exposure (Wang et al. 2013). Seafood generally contains organic arsenic forms (arsenobetaine, arsenocholine and arsenosugars) that have relatively low toxicity (Fattorini et al. 2004; Francesconi 2010; Jankong et al. 2007; Li et al. 2003; Nam et al. 2010; Schaeffer et al. 2006). However, a study in Cuba found that certain species of fish can accumulate extremely high levels of inorganic arsenic (over 98%), a more toxic form (Fattorini et al. 2004). This same study highlighted the importance of identifying the source of arsenic in marine environment, and the species of biota, as these are important factors that can determine the toxicity of arsenic.

These examples show the importance of seafood as a potential source of toxicity to human consumers. Fish and shellfish are important dietary components, particularly in coastal communities. These sources represent a rich source of protein, minerals, vitamins and essential fatty acids (Andreji et al. 2006). Nevertheless, they may be an important vector of toxicity.

1.5 REGULATORY STANDARDS

1.5.1 SEAWATER AND MARINE SEDIMENTS

Seawater and sediment quality guidelines have been developed by regulatory organisations to minimise adverse biological effects that chemical contaminants can have on the ecosystem. Such regulatory bodies include the National Oceanic and Atmospheric Administration (NOAA), and the Australia and New Zealand Environment and Conservation Council (ANZECC) (ANZECC 2000). There are four main protection levels (80, 90, 95 and 99%) identified in the ANZECC guidelines for seawater (Table 1.2). The 99% protection level indicates that a trace element concentration below that level will protect 99% of marine species against the onset of toxic effects of the given trace element. For marine sediments there are two protection levels (Table 1.2). They are the Interim Sediment Quality Guideline-low (ISQG-low) value, which is a trigger value for *possible* biological effect and the ISQG-high value, which indicates a *probable* biological effect.

Table 1.2: ANZECC trigger values for marine water (µg L^{-1}) at different levels of protection of marine species (% species), and ANZECC ISQG values for the protection of marine species (µg g dry wt^{-1}) for sediments

Trace element	ANZECC trigger values (µg L^{-1})				ANZECC (ISQG) values (µg g dry wt^{-1})	
	99%	95%	90%	80%	ISQG - low	ISQG - high
As	*	*	*	*	20	70
Cd	0.7	5.5	14	36	2	10
Cu	0.3	1.3	3	8	65	270
Fe	*	*	*	*	*	*
Pb	2.2	4.4	6.6	12	50	210
Zn	7	15	23	43	200	410

*** Values not provided in the ANZECC guideline.**

1.5.2 SEAFOOD

Limits for trace element concentrations in food standards are set to minimise health impacts on consumers, by limiting the intake of contaminated foods. In this regard, regulatory authorities include the World Health Organization (WHO), the European Commission (EC), the United States Food and Drug Administration (USFDA), and Food Standards Australia New Zealand (FSANZ). These agencies

provide guidelines for dietary intake limits and maximum allowable values of specific contaminants in food.

In the current thesis, results were compared with regulatory levels set by the FSANZ wherever possible (ANZFSC 2013), and to the EC regulations where FSANZ values were not available (Table 1.3). The exceptions to this were the maximum levels for copper (30 µg g wet wt^{-1}) and zinc (40 µg g wet wt^{-1}), which were obtained from the Food and Agricultural Organization (FAO) data for Australia and New Zealand other standards (Nauen 1983), as these elements were not included in FSANZ or EC regulations. These maximum levels of copper and zinc for fish and fish products also apply to crustaceans and molluscs. No limit was provided for iron in any of the food standards mentioned above.

Table 1.3: Trace element limits in regulatory standards for seafood

Trace elements	Maximum allowable levels (ML) (µg g wet wt^{-1})		
	FSANZ levels	Regulation EC. No 1881/2006, 420/2011	NZ/Australia other standards (FAO)
As (inorganic)			
Fish	2	-	
Crustacean	2	-	
Molluscs	1	-	
Cd			
Fish	-	0.05	
Crustacean	-	0.5	
Molluscs	2	1	
Hg			
Fish	0.5	0.5	
Crustacean	0.5	0.5	
Molluscs	0.5	0.5	
Pb			
Fish	0.5	0.3	
Crustacean	-	0.5	
Molluscs	2	1.5	
Cu			
Fish	-	-	30
Zn			
Fish	-	-	40

"-" indicates that no value is provided in the food standards. NZ- New Zealand. FSANZ- Food Standards Australia New Zealand.

Data for seafood trace element burdens collected in the current thesis were used to conduct risk assessments for the consumption of the species measured. Risk assessments compare the intake of trace elements to provisional tolerable weekly intake (PTWI) values. PTWI values are provided by the WHO/FAO Joint Expert Committee for Food Additives (JECFA) for inorganic arsenic, cadmium, methylmercury and lead, and are 21, 5.6, 1.6, and 25 μg/kg body weight/week, respectively (WHO 2000,2007,2010a,b).

1.6 ANALYTICAL METHODS FOR SEAWATER

Although an extensive number of studies have examined the analysis of the low concentrations of trace elements in marine systems, such analyses are still a challenge. This is largely because of the detection limit required, and matrix interferences (Anthemidis et al. 2011; Rahmi et al. 2007; Tuzen et al. 2005; Yuan et al. 2011). Seawater is a rich source of chemical elements including calcium, sodium, and organic and inorganic compounds. The concentrations of trace elements in seawater often lie below the detection limit of commonly used modern analytical instruments such as ICP-MS (Zhang et al. 2010). Furthermore, concentrated ions such as sodium and chloride in seawater samples can produce very complicated spectroscopic results for trace elements due to interferences (Brown & Milton 2005; Jenner et al. 1990; Korn et al. 2006). Thus, pre-concentration or separation procedures are needed to obtain an accurate result for trace level elements in complex matrices such as seawater (Daskalova & Boevski 1999).

A considerable amount of attention has been given to developing pre-treatment sample preparation methods (Zhang et al. 2010). These methods include liquid-liquid extraction, ion-exchange, electrochemical deposition, extraction chromatography, and solid phase extraction (SPE) (Zhang et al. 2010). Solid phase extraction methods are widely used due to their simplicity, multi-element enrichment, high selectivity, low cost, low solvent use, and the ability to couple them with different detection techniques (Tuzen et al. 2005; Zhang et al. 2010). Examples of SPEs used for trace elements include chemically modified silica gel, inorganic-organic hybrid materials like C18 silica cartridge, Chelex-100, Chromosorb resins, and functionalised styrene–

divinylbenzene copolymers (Camel 2003; Dakova et al. 2009; Hennion 1999; Zhang et al. 2010). Novel solid phases include animal fibrous proteins (AFPs) such as wool powder and silk powders, which are potentially useful solid phase extractants because of the many chemically active functional groups in these fibres that have high binding affinities for trace elements (Goto & Suyama 2000; Naik et al. 2010).

A weakness of trace element pre-concentration methods is that they often involve large volumes of seawater, high consumption of chemicals, and are time consuming (Naik et al. 2010). For these reasons alternative approaches are necessary. In this regard, iminodiacetate resins (SPR-IDA, suspended particulate reagent iminodiacetate) have been developed as solid phase treatments for pre-concentrating trace elements in seawater (CETAC 2011). The SPR-IDA suspension has been used to validate a method for extracting and pre-concentrating trace elements in seawater samples in this thesis. The solid phase SPR-IDA requires a small amount of the solid phase, is simple to use, and does not require a large volume of seawater unlike most of the pre-concentration methods currently employed.

1.7 STUDY SITES

This research project included fieldwork in two locations with distinct, yet related, sea-fill activities. They are Thilafushi Island in the Maldives (MSW sea-fill for reclamation) and Lyttelton Harbour, New Zealand (clean construction rubble from earthquake damaged buildings for reclamation).

1.7.1 THILAFUSHI ISLAND OF MALDIVES

Thilafushi Island is an artificial island created through waste disposal in a lagoon called ‘Thilafalhu’, located 6.85 km northwest of Male’, the Maldives capital. The lagoon is 7 km long and approximately 200 m wide, and located in very close proximity to other inhabited islands. The lagoon is still in the process of reclamation using different types of solid waste and dredged sand from the inner lagoon (CDE Consulting 2011). Thilafushi Island is the main waste disposal site for Male’ and surrounding islands, including several tourist resorts. The MSW disposed includes domestic and industrial waste of organic and inorganic origin. The size of this waste

also varies, ranging from small tins to whole cars (Khaleel & Saeed 1997). Solid waste disposed of at Thilafushi Island is known to contain used batteries, asbestos, paints, all types of metals, e-waste and other potentially hazardous chemicals (UNEP 2005b). Leaching of trace elements from Thilafushi Island into the lagoon could be a potential health hazard as local people consume fish and seafood harvested near the lagoon. No investigation quantifying trace elements at Thilafushi Island of Maldives had been conducted prior to the start of this project.

1.7.2 LYTTELTON HARBOUR OF NEW ZEALAND

Lyttelton Harbour is located adjacent to the New Zealand city of Christchurch. It is situated on the east coast of the South Island, and it is the northern major sea inlet formed by an enclosed rock-wall on Banks Peninsula (ECan 2008; Hart 2004). Banks Peninsula was once a volcanic island, and Lyttelton Harbour is the flooded crater of a volcano that erupted millions of years ago (Liggett & Gregg 1965; Stipp & McDougall 1968). The Lyttelton port lies about 4.5 km from the head entrance of the harbour. The harbour is approximately 15 km long and on average 2 km wide (Hart 2004). After the Canterbury Earthquake in 2011, Lyttelton Port of Christchurch (LPC) Ltd. were granted approval by the Canterbury Regional Council (Environment Canterbury; ECan) and Christchurch City Council (CCC) to use "clean" earthquake rubble, which included stone, bricks, tiles, aggregates, reinforced and unreinforced concrete, general rubble, glass and cured asphalt (LPC 2011), to reclaim ten hectares of Lyttelton Harbour (Te Awaparahi Bay) (LPC 2014). Cured asphalt was only allowed to be placed out of the wave erosion zone. The sea-fill was created adjacent to the Port at Te Awaparahi Bay, between the Cashin Quay breakwater and Battery Point.

1.8 RESEARCH OBJECTIVES

1.8.1 AIMS

This research aimed to assess the level of trace element contamination at the sea-fill sites of Thilafushi Island, Maldives, and Lyttelton Harbour, New Zealand.

The specific objectives of the research were to:

- Validate suitable and simple methods for analysis of trace elements in seawater, marine sediment and marine biota

- Characterise the trace element concentrations in seawater, sediment and biota from the vicinity of Thilafushi Island sea-fill, and to prepare a risk assessment for consumption of seafood from the vicinity of the sea-fill site in Thilafushi Island

- Monitor trace element concentrations in seawater, sediment and biota from the vicinity of the sea-fill at Lyttelton Harbour of New Zealand

- Investigate trophic transfer of trace elements in the coastal food chain of Lyttelton Harbour

- Undertake a baseline study of trace element concentrations in fish and shellfish, and prepare a risk assessment for consumption of seafood from Lyttelton Harbour

1.9 THESIS STRUCTURE

This thesis is presented in seven chapters, including this introduction chapter.

Chapter 2: Describes the analytical methods for determining the concentration of selected trace elements in seawater, marine sediment and biota samples. Method validation is presented in this chapter.

Chapter 3: This chapter presents the results for trace element concentrations in seawater, sediment and biota in the vicinity of the sea-fill site of Thilafushi Island of the Maldives and the risk assessment for consumption of seafood.

Chapter 4: The monitoring results for trace elements in seawater, marine sediments and green-lipped mussels at the sea-fill site of Lyttelton Harbour are provided in this chapter.

Chapter 5: This chapter contains the results for trace element concentrations in marine biota in a coastal food chain of Lyttelton Harbour, along with the risk

assessment for consumption of fish harvested from Lyttelton Harbour

Chapter 6: The results of the baseline study of trace elements in three species of shellfish from Lyttelton Harbour are presented in this chapter, along with the risk assessment for the consumption of wild shellfish from the harbour bays.

Chapter 7: Overall conclusions and recommendations for future work are presented in the final chapter.

CHAPTER 2

MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter describes the materials and methods used to obtain the results presented in this thesis. Three types of environmental matrices were collected and analysed: seawater, marine sediment and marine biota. A solid phase extraction (SPE) method was used to extract trace elements from seawater samples. Suspended particulate reagent iminodiacetate (SPR-IDA) was used as the solid phase for pre-concentrating trace elements. The SPE method used in this thesis was adopted from the guidelines of CETAC Technologies (CETAC 2011) and validated for this work. Acid digestion methods were used to extract trace elements from marine sediments and biota. All samples were prepared and analysed at the University of Canterbury. Analyses for trace elements were carried out by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS), except for iron in marine sediment samples, which was analysed by Atomic Absorption Spectrometry (AAS). Details of fieldwork and methods for sample collection are provided in the relevant thesis chapters (Chapters 3-6).

2.2 CHEMICALS AND MATERIALS

All containers and vials used in this study were plastic to minimise any trace element contamination. Acid-cleaned polycarbonate vials (50 mL) were used in the digestions of marine sediments and biota samples. Similarly, acid-cleaned polypropylene vials (50 mL and 15 mL) were used in the seawater collection and extraction work. Custom-built aluminium hot plates were used for digesting all sediments and biota samples. Acid-cleaned plastic jars were used to collect sediments, while acid-cleaned vials and snap-lock plastic bags were used for collecting and transporting biota from field to laboratory.

2.2.1 CHEMICALS AND MATERIALS FOR SEAWATER ANALYSES

The solid phase used for extracting trace elements in seawater in this study was a chelating polymer resin reagent, SPR-IDA. This consists of a polystyrene reagent bead of 10 microns (0.01 mm) and was purchased as a 10% suspension (w/v) in deionised water, from CETAC Technologies. The ultrapure (70%) nitric acid, and ultrapure (24%) hydrochloric acid used in the extraction work were sub-boiled quartz distilled acids purchased from the University of Otago, New Zealand. The ammonium hydroxide solution (21%, w/w Optima, for ultra trace analysis) was purchased from Fisher Scientific UK. Yttrium stock solution (10,000 ppb in 2% HNO_3), mixed elements (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) stock solution (10,000 ppb in 2% HNO_3), mercury stock solutions (10,000 ppb in 2% HNO_3), along with other ICP-MS standards, were purchased from Inorganic Ventures. Sodium chloride (analytical grade) was purchased from LabServ, Biolab, Australia Ltd., and the certified reference seawater (NASS-6, NRC, National Research Council of Canada) purchased from the National Research Council of Canada.

2.2.2 CHEMICALS AND MATERIALS FOR MARINE SEDIMENT ANALYSES

The acids used in the digestion process for extracting trace elements in marine sediments were analytical grade nitric acid (69%), and analytical grade hydrochloric acid (34%) purchased from Merck, Germany. However, ultrapure acids (with 0.1% L-cysteine added) were used for diluting the digests of marine sediments. The diluting

acid mixture strengths were 2% nitric acid and 0.5% hydrochloric acid. Sigma L-cysteine (analytical grade; to ensure mercury retention in solution) from a non-animal source was used in preparing the ultrapure acid mixture. Marine sediment SRM-2702 (NIST; National Institute of Standards and Technology) was used as a certified reference material. Single element iron stock solution (1000 mg L^{-1}) purchased from Merck, Germany was used to prepared the calibration standards for sediment iron analysis by AAS.

2.2.3 CHEMICALS AND MATERIALS FOR BIOTA

The acids used in extracting trace elements from biota were ultrapure nitric acid (70%) and ultrapure hydrochloric acid (24%), as for seawater extractions. Ultrapure acids (with L-cysteine added) were used for diluting the digests of marine biota (same acid mixture as that used for sediment dilution). Certified reference materials included mussel tissue (SRM-2976, EVISA, European Virtual Institute for Speciation Analysis), fish protein (DORM-3, NRC, National Research Council of Canada), bovine liver (SRM 1557c, NIST, National Institute of Standards and Technology) and tomato leaves (SRM 1573a, EVISA, European Virtual Institute for Speciation Analysis).

2.3 ANALYSIS OF TRACE ELEMENTS IN SEAWATER

Seawater samples from the Maldives were acidified to pH 2 using ultrapure nitric acid within 15 days of sample collection, and after importation to New Zealand due to restrictions on transporting acidified samples. These seawater samples were acidified to prevent the trace elements from adsorbing onto the surface of the vials and reverse any adsorption that had already occurred. Seawater samples from the Maldives were extracted within 3 months of collection.

All seawater samples from New Zealand were acidified to pH 2 with ultrapure nitric acid within 12 to 20 hours of sample collection. Seawater samples from New Zealand were extracted within 48 hours of sample collection. All seawater samples were stored at 4°C until solid phase extraction, and all were extracted using the method adopted from the manufacturer of the solid phase reagent (CETAC 2011), as

described in Section 2.3.1.

Seawater samples (15 mL) were extracted for trace elements (cadmium, copper, iron, lead and zinc) using SPR-IDA as the solid phase (CETAC 2011). All field blanks (one vial of 50 mL Milli-Q water for each of the transect lines) were also extracted along with their respective environmental seawater samples. Trace elements spiked in natural seawater, Milli-Q water and artificial seawater (freshly prepared) were also extracted (with their blanks) with each batch (n = 30-35) of environmental seawater. Artificial seawater was prepared with analytical grade sodium chloride salt and Milli-Q water at the concentration of 19.37 g/kg, (salinity of approximately 35.5‰) as this is the concentration of sodium chloride reported for natural seawater (Huber et al. 2000). Comparative standards were prepared for each level of trace element spike to calculate the percentage recovery of spiked trace elements in these different types of water samples.

2.3.1 EXTRACTION PROCEDURE FOR SEAWATER

SPR-IDA suspension (100 µL) was added to the water samples (15 mL), and the internal standard yttrium was spiked at a concentration of 250 µg L^{-1}. The pH of the samples was adjusted to 8 using ammonium hydroxide solution (21%, w/w Optima). Samples were gently but thoroughly mixed, and left to settle for one hour, and thereafter were centrifuged for 15 min at 4500 RPM (revolutions per minute). The supernatant was then decanted, and the solid phase was washed with Milli-Q water (15 mL) of pH 8 (pH adjusted with the ammonium hydroxide solution). This step removed interfering ions such as sodium, magnesium and calcium without permitting the leaching of the solid phase-bound trace elements (CETAC 2011). For washing the solid phase, Milli-Q water (pH 8) was added to the solid phase, mixed and left to settle for one hour. The samples were then centrifuged and the supernatant was decanted. Elution was carried out with 7% ultrapure nitric acid. Nitric acid (1.5 mL) was added to the solid phase, mixed well and left to settle for one hour, before centrifugation. This step was then repeated and the two resulting aliquots were collected, mixed well and analysed by ICP-MS. A schematic diagram of the solid phase extraction steps showing interactions of trace elements with the solid phase is presented in Figure 2.1.

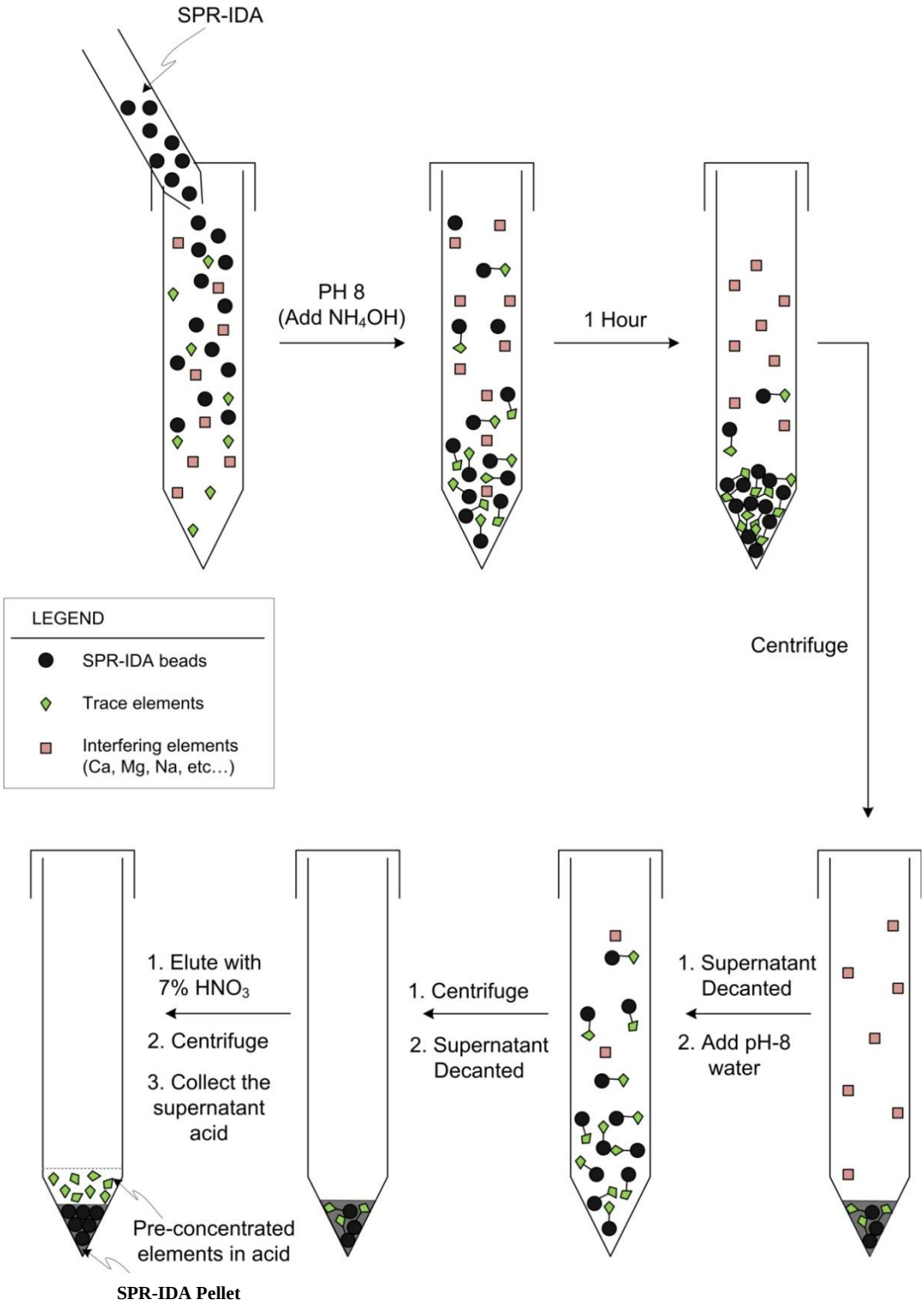


Figure 2.1: Schematic diagram of solid phase extraction concept showing the interactions of solid phase and the trace elements of interest during the extraction process

2.3.2 QUALITY ASSURANCE (QA) / QUALITY CONTROL (QC)

2.3.2.1 CLEANING AND PREPARATION OF EQUIPMENT

All vials and containers were acid-cleaned prior to use for sample collection and preparation. Extra care was taken in acid-cleaning materials used for seawater collection as seawater was expected to contain very low concentrations of these elements, and thus even a trace level of contamination could impact results. Therefore all containers and vials were acid washed for 5 days in 10% nitric acid followed by 2% nitric acid for another 5 days. These containers were then rinsed with Milli-Q water six times, and air dried in a clean (metal-free) room. Acid-cleaned polypropylene vials (50 mL) were used for collecting seawater samples and 15 mL vials were used for the extractions. Following collection of seawater, the samples were kept in small snap-locked plastic bags (each vial in a separate bag) to avoid any cross contamination, and stored at 4°C until extracted. All extractions were carried out in a metal-free clean room, except for the centrifugation step, wherein samples were taken to the centrifuge location in a well-sealed clean cooler to prevent contact with outside dust. Maldives seawater samples were extracted in a fume cupboard in a physical containment level 2 (PC2) laboratory for bio-security reasons.

2.3.2.2 QUALITY ASSURANCE MEASURES

In the processing of seawater samples, every 10^{th} sample was extracted in duplicate to assess the accuracy of the extraction process. Every extraction batch included four field blanks as mentioned earlier (one for each transect line). Here, the field blanks were Milli-Q water samples (50 mL) carried to the field, and exposed to the environment to determine any incidental contamination from environmental conditions. In addition to the field blanks, each batch of extracted samples included at least two levels (1 µg L^{-1} and 10 µg L^{-1}) of trace element spikes in natural seawater, artificial seawater and Milli-Q water. Every other extraction batch was carried out with a third level of spike in each type of water (5 µg L^{-1}). Duplicates of trace element spiked samples were also extracted in every other extraction batch (for example, duplicates of the 1 µg L^{-1} or 10 µg L^{-1} or 5 µg L^{-1}). Each type of trace element-spiked water sample was extracted with its blank for checking any contamination during the extraction process. A comparative standard was prepared with the eluting acid (7%

ultrapure nitric acid, to match the acid strengths of the samples) for each level of trace element spike in the QA water samples above. The comparative standards were spiked at the same time as the QA water samples to ensure exactly the same concentrations of trace elements were spiked. Comparative standards were used to calculate recoveries of trace elements from spiked samples. A certified reference seawater sample (NASS-6, NRC) was also extracted with each batch of seawater. When trace element concentrations in the field blanks were higher than the instrument detection limits, a blank correction was applied to the final results. All duplicates were averaged to obtain a final result.

2.3.3 METHOD VALIDATION FOR SEAWATER EXTRACTION

Method validation was carried out for four different types of water samples (freshly prepared artificial seawater, natural seawater, Milli-Q water and certified reference seawater (NASS-6, NRC)). Fifteen mL aliquots of these waters were extracted using SPR-IDA as the solid phase, as described in Section 2.3.1. With the exception of the NASS-6 seawater, all other water samples were spiked at 5 µg L^{-1}. A blank for each type of water (Milli-Q water blank for NASS-6) was also extracted alongside the spiked samples, and a comparative standard was prepared from the eluting acid (7% ultrapure HNO_3) at a trace element concentration of 5 µg L^{-1}. The percentage recoveries of the spiked trace elements in the water samples, and the percentage recoveries of trace elements in the NASS-6 seawater, are presented in Table 2.1.

Table 2.1: Percentage recovery of the 5 µg L^{-1} spiked trace elements in three water types and the trace elements in the certified reference water (NASS-6) after blank correction (n=2)

Analytes	Milli-Q water	Artificial seawater	Natural seawater	NASS-6 seawater
Cadmium	95.3	95.2	98.6	96.4
Copper	94.1	98.5	90.5	114.2
Iron	79.5	94.5	119.1	<LOD
Lead	89.3	85.9	80.7	76.2
Zinc	112.6	103.3	89.4	97.7

To determine the reliability and reproducibility of the extraction method, a series of experiments with different levels of trace element spikes (1, 5, and 10 µg L^{-1})

were carried out. This included duplicates of spiked natural seawater, freshly prepared artificial seawater and Milli-Q water for each spike level (i.e. n = 6 for each type of water). In addition, 6 samples of certified reference seawater (NASS-6, NRC) were extracted alongside the trace element-spiked water samples. Blanks and comparative standards were also included for each spiked level for calculating percentage recoveries (comparative standards provided the exact values of the trace elements that were spiked in the samples). Any blank contribution was corrected (to eliminate any pre-existing trace elements in the samples or any contribution from sample handling) before the percentage recovery calculations. The percentage recoveries of trace elements in NASS-6 seawater are presented in Table 2.2, and the percentage recoveries of the spiked trace elements in the water samples are presented in Table 2.3.

The iron concentration in NASS-6 reference seawater was below the limit of detection (LOD). The percentage recovery of lead in NASS-6 water was more variable than for the other elements (the %RSD for lead was 11.5%). The % RSD for cadmium, copper, iron and zinc was always below 10%.

Table 2.2: Percentage recoveries and statistical summary of trace elements extracted from the certified reference seawater (NASS-6, NRC)

Analyte	Mean % recovery (n=6)	Std dev	%RSD	95% C.I.
Cadmium	98.1%	3.1%	3.1%	2.5%
Copper	101.1%	6.7%	6.6%	5.4%
Iron	<LOD	-	-	-
Lead	83.3%	9.5%	11.4%	7.6%
Zinc	93.3%	4.2%	4.5%	3.3%

Std dev – standard deviation, **%RSD** – percentage relative standard deviation, **95%C.I.** – 95% percent confidence interval.

Table 2.3: Mean percentage recoveries and statistical summary of spiked trace elements in three different types of water

Analyte	1 µg L^{-1} spike (n = 6)				5 µg L^{-1} spike (n = 6)				10 µg L^{-1} spike (n = 6)			
	Mean % recovery	Std dev	%RSD	95% C.I	Mean % recovery	Std dev	%RSD	95% C.I.	Mean % recovery	Std dev	%RSD	95% C.I.
Milli-Q water												
Cadmium	90.4%	2.4%	2.7%	1.9%	93.5%	3.5%	3.7%	2.8%	93.4%	3.1%	3.3%	2.5%
Copper	105.1%	3.9%	3.7%	3.3%	93.6%	5.3%	5.6%	4.2%	98.8%	7.5%	7.6%	6.0%
Iron	82.4%	7.9%	9.6%	6.3%	87.5%	9.3%	10.6%	7.4%	89.8%	8.6%	9.6%	6.9%
Lead	93.2%	2.8%	3.0%	2.2%	91.1%	8.8%	9.6%	7.0%	91.3%	3.6%	3.9%	2.9%
Zinc	87.8%	6.8%	7.8%	5.5%	95.9%	10.5%	11.0%	8.4%	92.0%	4.0%	4.4%	3.2%
Artificial seawater												
Cadmium	93.5%	3.0%	3.2%	2.4%	92.4%	4.3%	4.7%	3.5%	96.5%	4.6%	4.7%	3.7%
Copper	93.2%	6.2%	6.6%	4.9%	92.8%	7.0%	7.5%	5.6%	97.5%	7.1%	7.2%	5.6%
Iron	98.9%	15.7%	15.8%	12.5%	87.2%	4.8%	5.5%	3.9%	93.6%	11.7%	12.5%	9.4%
Lead	87.3%	2.3%	2.6%	1.8%	90.1%	8.2%	9.1%	6.6%	87.9%	7.0%	7.9%	5.6%
Zinc	97.5%	8.6%	8.8%	6.9%	96.7%	12.3%	12.7%	9.8%	101.2%	5.2%	5.3	4.3
Natural seawater												
Cadmium	96.2%	3.7%	3.8%	2.9%	105.1%	1.9%	1.8%	1.5%	99.2%	5.7%	5.7%	4.5%
Copper	92.9%	6.4%	6.9%	5.1%	102.6%	1.6%	1.5%	1.3%	93.8%	6.4%	6.8%	5.1%
Iron	<LOD	<LOD	<LOD	<LOD	95.5%	10.1%	10.5%	8.0%	103.0%	14.3%	13.9%	11.4%
Lead	86.6%	3.9%	4.5%	3.1%	82.7%	1.1%	1.3%	0.9%	88.1%	2.6%	2.9%	2.0%
Zinc	99.8%	10.8%	10.9%	8.8%	104.2%	2.6%	2.6%	2.2%	101.1%	6.4%	6.4%	5.1%

LOD – Limit of detection, **Std dev** – standard deviation, **%RSD** – percentage relative standard deviation, **95%C.I.** – 95% confidence interval.

The mean percentage recoveries of cadmium, copper and zinc were always greater than 90% in Milli-Q water for all three levels of spikes (Table 2.3), with iron and lead showing a slightly lower percentage recovery (always above 82% in all water types for the spike levels 5 and 10 µg L^{-1}). The limit of detection (LOD) for iron was higher than 1 µg L^{-1} and the ICP-MS measurements for 1 µg L^{-1} comparative standards were variable. Spike recovery of iron and zinc showed more variability compared to the other elements. Higher levels of iron and zinc were also observed in the blank extracts compared to other elements. This could be due to their high abundance in the environment and laboratory. Building repair work was being carried out in the Chemistry building at the time of these extractions, and although the samples were tightly sealed when removed from the clean room for centrifugation, this may still have contributed to some contamination.

2.3.4 LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The LOD and LOQ values were determined using the United States Environmental Protection Agency (USEPA) recommended guideline (USEPA 2005), and are presented in Table 2.4. The LOQ's were determined using low level trace element-spiked natural seawater, unspiked natural seawater, and spiked and unspiked Milli-Q water extractions (n = 7). Natural seawater for this extraction was sourced off the coast of Pigeon Bay of Banks Peninsula, New Zealand. Seven replicates of the natural seawater and Milli-Q water were spiked at 5 µg L^{-1} concentrations, which was approximately five times the estimated detection limit of the ICP-MS used for this analysis. The seven unspiked seawater samples were processed to account for any matrix contribution. The limit of detection (LOD) was 3 times the standard deviation of the unspiked Milli-Q water and natural seawater samples (Table 2.4). The LOQ was calculated by multiplying the standard deviation of the seven spiked replicates by the student t-value at $\alpha = 0.01$ (3.14). These statistically derived LOQ's for the analytes in seawater were higher than the actual solution concentrations that could be achieved for all analytes in seawater, and therefore lower practical quantitation limits were applied. The applied practical LOQ's were estimated using the standard deviation values of the seven unspiked seawater and Milli-Q water replicates, as well as checking the precision of the lowest standards used in the analysis. The percentage

difference (<10%) of the low concentration trace elements in the duplicate samples was also taken into account.

Table 2.4: LOD and LOQ for trace elements in seawater (µg L^{-1})

Analyte	Calculated LOD for Milli-Q water	Calculated LOD for seawater	Calculated LOQ for Milli-Q water	Calculated LOQ for seawater	Estimated and applied practical LOQ
Cadmium	0.02	0.01	0.26	0.22	0.04
Copper	0.22	0.07	0.34	0.61	0.29
Iron	1.95	13.40	0.78	17.85	2.68
Lead	0.05	0.04	0.21	0.19	0.04
Zinc	0.60	0.34	1.24	1.70	1.18

LOD – Limit of detection**, LOQ** – limit of quantification

2.4 METHODS FOR SEDIMENT AND BIOTA ANALYSIS

Detailed methods for sediment and biota collection, and pre-treatment of samples, are provided in the respective chapters. Details of the acid digestion, dilution and analytical methods are provided in this chapter.

2.4.1 SAMPLE PREPARATION AND DIGESTION

2.4.1.1 MARINE SEDIMENTS

Total recoverable concentrations of arsenic, cadmium, copper, iron, lead, mercury and zinc in marine sediments were determined using an acid digestion method adapted from USEPA Method 200.2. Upon collection, the sediment samples were placed on ice until transported to the laboratory. Sediment samples were then transferred to aluminium trays and oven dried at 30°C for 7-10 days. The dried sediments were then transferred to plastic snap-lock bags and crushed within the bags using a stainless steel iron bar. Sediments were sieved to less than 2 mm using stainless steel sieves. Individual samples were stored in separate snap-lock plastic bags at room temperature until acid digested.

Oven-dried (30°C, < 2 mm, 1 g) samples were weighed into acid-cleaned polycarbonate vials (50 mL). Analytical grade nitric acid (4 mL, 50%) and analytical grade hydrochloric acid (10 mL, 20%) were added to the samples. The samples were gently mixed, left for one hour, and then digested at 85°C for another hour under continuous refluxing. The digests were cooled overnight in a fume hood at room temperature and diluted to 20 mL with Milli-Q water. Each sample was again diluted twenty one times (0.5 mL of digest plus 10 mL diluting acid mixture (2% HNO_3/0.5% HCl/0.1% L-cysteine)), and analysed for arsenic, cadmium, copper, mercury, lead and zinc by ICP-MS (Agilent 7500cx ICP-MS ASX-500 auto sampler). Iron was analysed by flame AAS (Varian SpectrAAS220FS). A procedural blank, internal quality assurance samples (certified reference marine sediment, SRM-2702, NIST), and duplicates (one per 10 samples) were included in each batch of sediment digestion.

2.4.1.2 MARINE BIOTA

Five groupings of biota samples were analysed (green algae, marine worm, bivalve, crab and fish). All biota samples were freeze-dried (-40°C at < 0.133 mBar vacuum) (Figure 2.2A) for 7-10 days, except green algae from the Maldives (oven dried at 50°C for 5-7 days due to restrictions in bringing fresh algae to New Zealand). The freeze-dried biota samples were homogenised using a small stainless steel spice grinder.

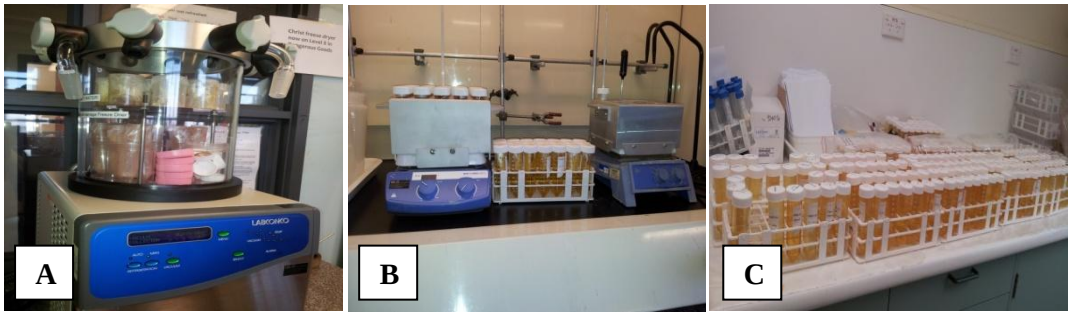


Figure 2.2: Main steps included in the biota sample preparation and digestion

Homogenised biota samples (200 mg) were weighed into acid-cleaned polycarbonate centrifuge vials (50 mL), and to these samples, concentrated nitric acid (1 mL, 70% ultrapure), and concentrated hydrochloric acid (0.2 mL, 24% ultrapure) were added. These samples were mixed gently, left for at least one hour, and digested

at 85°C for another 1 hour under continuous refluxing (Figure 2.2B) on a hot plate. Samples were cooled by leaving them overnight at room temperature in a fume hood, before they were made up to 20 mL by adding Milli-Q water and mixed gently (Figure 2.2C). Each sample was then diluted five times (1 mL of digest plus 4 mL of dilute acid (2% HNO_3/0.5% HCl/0.1% L-cysteine)), and analysed for arsenic, cadmium, copper, iron, mercury, lead and zinc by ICP-MS. A procedural blank, internal quality control (a certified reference material varying according to the type of biota), and duplicates (one per 10 samples) were included in each batch of biota digestion.

2.4.2 QUALITY ASSURANCE / QUALITY CONTROL

All vials and materials were acid-cleaned in 10% nitric acid for 5 days and rinsed three times with Milli-Q water. These vials were then air dried in a clean room prior to use. Acid-cleaned plastic spoons were used in transferring samples during the weighing processes. Spoons were cleaned with methanol (HPLC grade) between samples. Acid-cleaned pipette tips were used for transferring acids. All dilutions were carried out in fume hoods in a metal-free clean room to prevent contamination. The sieves used for separating < 2 mm sediment were washed thoroughly with distilled water, then rinsed with Milli-Q water, and dried in an oven at 100°C between samples to avoid any cross-contamination.

Biota samples were freeze-dried prior to homogenisation. Freeze drying was preferred over oven drying to prevent contamination, as well as to preserve mercury species in the biota samples (LaFleur 1973), as mercury can be volatilised from the sample at temperatures above 60°C (Pillay et al. 1971). The biota samples were homogenised using a stainless steel spice grinder, and the spice grinder was cleaned with methanol (HPLC grade) between the samples to avoid cross-contamination.

All reagents were freshly prepared before digestion. Certified reference samples were prepared and digested alongside the environmental samples for quality assurance. For each batch of samples (n = 20 for sediments; n = 12-24 for biota) that were placed together on a hotplate, at least one procedural blank was included. Every 10^{th} sample of sediment and biota was digested in duplicate for checking the consistency of the digestion process.

Standard reference marine sediment (SRM-2702, NIST) was included in duplicate with each batch of sediment digestions. Appropriate standard reference materials according to the type of biota species (SRM-2976 - mussels; DORM-3 - fish protein; SRM 1557c - bovine liver; and SRM 1573a - tomato leaves) were also included with every batch of biota digestion as quality assurance samples. All the results were reported after correcting any blank contribution higher than the applied detection limits for the respective sample type. The average of the 10^{th} sample duplicates was calculated, and this value was the reported result for the duplicate samples. The percentage differences of the duplicates were always below 10% for the samples with concentrations higher than the applied practical LOQ, and for sediments most of the time this difference remained below 5%.

Sample dilutions included one duplicate for every 10^{th} sample, and one triplicate for every 20^{th} sample. The duplicate dilutions were to check instrument efficiency during the analysis. The triplicate samples were spiked with a known concentration of trace elements, and the percentage recoveries were determined to verify whether there were any interferences or losses occurring during the analysis.

2.4.3 METHOD VALIDATION

2.4.3.1 MARINE SEDIMENTS

One gram of the homogenised standard reference marine sediment (SRM-2702, NIST) was digested in duplicate along with an acid blank. The samples were weighed into 50 mL polycarbonate vials and digested in 10 mL of nitric acid (analytical grade, 50%) and 4 mL of hydrochloric acid (analytical grade, 20%) at 85°C for 1 hour under continuous refluxing. The samples were left to stand for at least one hour after addition of the acids, before placing them on the hotplate. Hydrochloric acid was used in the digestion to retain any mercury species in the samples (Louie et al. 2012). Samples were cooled by leaving overnight at room temperature in a fume hood. These digests were made up to 20 mL by adding Milli-Q water, mixed gently, and left to settle overnight. Each sample was then diluted twenty one times (0.5 mL of digest plus 10 mL of diluting acid mixture (2% HNO_3 and 0.5% HCl with 0.1% L-cysteine)), and analysed for arsenic, cadmium, copper, mercury, lead and zinc by ICP-MS. L-cysteine was included in the acid mixture to prevent mercury adsorption on the

wall of the vial, and to keep mercury in solution, owing to the ability of L-cysteine to complex strongly with mercury (Li et al. 2006; Wang et al. 2010).

Sediment iron was analysed by flame AAS, due to its high concentration compared to the other trace elements in the sample, while all other elements were analysed by ICP-MS. Percentage recoveries of over 90% for all the tested trace elements were achieved. To determine the reliability and reproducibility of the acid digestion method, five different sets of SRM-2702, in duplicate, were digested on five different days along with acid blanks. The average percentage recoveries of trace elements in the standard reference material (n = 10) were greater than 90% for all elements (Table 2.5). The %RSDs for all trace elements were less than 7% with most being less than 5%.

Table 2.5: Percentage recoveries and statistical summary of the trace elements in marine sediment (SRM-2702, NIST, mg kg^{-1})

Standard Reference Material of Marine Sediment (SRM-2702, NIST), n=10						
Analyte	**Certified SRM values**	**Measured SRM values**	**Mean % Recovery**	**Std dev**	**%RSD**	**95% C.I.**
Arsenic	45.3 ± 1.8	45.4 ± 2.2	100.3%	4.9%	4.9%	3.0%
Cadmium	0.817 ± 0.011	0.874 ± 0.044	106.9%	5.4%	5.0%	3.3%
Copper	117.7 ± 5.6	106.1 ± 5.4	90.1%	4.4%	4.9%	2.7%
Iron*	7.91 ± 0.24 %	7.30 ± 0.19 %	92.3%	3.3%	3.6%	2.1%
Lead	132.8 ± 1.1	121.9 ± 5.8	91.8%	4.4%	4.8%	2.7%
Mercury	0.4474 ± 0.0069	0.4070 ± 0.0275	91.0%	6.2%	6.8%	3.8%
Zinc	485.3 ± 4.2	442.7 ± 21.8	91.2%	5.5%	6.0%	3.4%

*Certified value of iron in the SRM-2702 was provided in % weight.

2.4.3.2 MARINE BIOTA

Two hundred milligrams of the homogenised mussel standard reference material (SRM-2976, EVISA), fish protein (DORM-3), bovine liver (SRM 1557c, NIST) or tomato leaves (SRM 1573a, EVISA) were digested in duplicate along with acid blanks. These samples were digested in 1 mL of concentrated nitric acid (ultrapure, 70%) and 0.2 mL of concentrated hydrochloric acid (ultrapure, 24%) at 85°C for 1 hour under continuous refluxing. The samples were left for at least one hour after addition of the acids, before placing them on the hotplate. As for marine sediments, hydrochloric acid was used to retain any mercury species in the samples (Louie et al. 2012). Samples were cooled by leaving them overnight at room

temperature in a fume hood and then were made up to 20 mL by adding Milli-Q water and mixed gently. Each sample was then diluted five times (1 mL of digest plus 4 mL of the diluting acid (same acid mixture as sediment)), and analysed for arsenic, cadmium, copper, iron, mercury, lead and zinc by ICP-MS.

The percentage recoveries of all trace elements in the standard reference mussel and fish protein were over 90% (except lead in DORM-3, which was 34%). Percentage recoveries in bovine liver were over 90% for all elements. For tomato leaves all elements were over 90%, with the exception of arsenic and mercury. Arsenic and mercury were <LOQ in the tomato leaves. To determine the reliability and reproducibility of the acid digestion method for biota, more sets of these standard reference materials were digested (as described above) in duplicate with acid blanks on different days. The percentage recoveries of the trace elements in standard reference mussel and fish protein are presented in Table 2.6, and that of bovine liver and tomato leaves are presented in Table 2.7.

The percentage recoveries of the trace elements in the certified reference mussel tissue (SRM-2976) were greater than 90% in all the runs for all elements with the exception of mercury (Table 2.6). Although the mean percentage recovery of mercury in SRM mussel (n = 19) was 93%, it ranged between 80 to 114% in different analytical runs. The %RSDs for all elements were below 10%, except for mercury which was 13.9%.

The mean percentage recovery of the trace elements in the certified reference fish protein (DORM-3) was above 94% for all elements except lead, which ranged between 32-42% (Table 2.6). It was observed that there were oily solid residues in the digests of DORM-3, which may have been resistant to the digestion method employed. Initially it was thought that the lead may be bound in these solid residues. Therefore digestion was carried out including hydrogen peroxide (30% analytical grade). This method achieved complete digestion of all solid materials in the sample, leaving a clear digest solution. However, the analytical result for lead recovery remained similar (39.7%). To determine if there were any matrix interferences, duplicate samples were digested using the normal digestion method after dry DORM-3 samples (duplicate) were spiked with mixed trace elements (20 μL of 10,000 ppb stock solution). The recovery for the spiked lead was 110% (with only a 5%

difference in the duplicate samples) indicating that the low recovery of lead in the DORM-3 fish protein was unlikely to be due to a matrix effect. The digests were also diluted 2, 5 and 10 times and analysed for any enhanced recovery. All dilutions resulted in 33-38% recovery. Problems with trace element recovery and complete digestion of DORM-3 reference material have been previously reported (Ashoka et al. 2009). Undigested dry DORM-3 samples were then sent to a commercial analytical laboratory (Hills Laboratory, Hamilton, NZ) to validate the results, and these analyses determined similar recoveries to those found in the current study, indicating that the DORM-3 is not a suitable reference material for lead analysis. The %RSD's for all the elements analysed, including lead, were below 10% for the DORM-3 samples.

The average percentage recovery of the trace elements in the bovine liver standard reference material (SRM 1577c) were over 93% for all trace elements except for arsenic (Table 2.7), which was <LOD. The %RSDs for all trace elements except lead (11%) were below 10%.

The average percentage recovery of trace elements in the tomato leaves standard reference material (SRM 1573a) was above 91% for cadmium, copper, iron and zinc (Table 2.7). Arsenic and mercury were <LOQ in the standard reference tomato leaves samples. The certified value for lead in tomato leaves (SRM 1573a) was not provided. The spiked (20 µg L^{-1}) recovery of arsenic in 5-times and 10-times diluted samples resulted in slightly higher (125-130%) recovery than the normally accepted range (80 -120%). The low level spiked recovery of mercury (0.24 µg L^{-1}) in the sample solution was 103% while the spiked (2 µg L^{-1}) recovery for lead was 91%, indicating that the arsenic, mercury and lead can be analysed accurately with this method. The %RSDs for all elements were below 8%.

Table 2.6: Percentage recoveries and statistical summary of trace elements in mussel tissue and fish protein standard reference material

Trace elements	SRM certified values	SRM values of this work	Mean % Recovery	Std dev	%RSD	95% C.I.
Mussel Standard Reference Material (SRM-2976, EVISA, mg kg^{-1}) n = 19						
Arsenic	13.3 ± 1.8	15.33 ± 0.7	115.2%	4.8%	4.2%	2.2%
Cadmium	0.82 ± 0.16	0.87 ± 0.04	105.5%	4.8%	4.5%	2.1%
Copper	4.02 ± 0.33	3.72 ± 0.16	92.5%	4.1%	4.4%	1.8%
Iron	171 ± 4.9	170 ± 9.7	99.5%	5.6%	5.7%	2.5%
Lead	1.19 ± 0.18	1.20 ± 0.12	100.9%	9.9%	9.8%	4.4%
Mercury	0.061 ± 0.004	0.057 ± 0.008	93. 5%	12.9%	13.9%	5.8%
Zinc	137 ± 13	139.57 ± 6.57	101.9%	4.8%	4.7%	2.2%
Fish Protein Standard Reference Material (DORM-3, mg kg^{-1}), n = 10						
Arsenic	6.88 ± 0.3	6.77 ± 0.3	97.1%	4.2%	4.3%	2.6%
Cadmium	0.290 ± 0.02	0.288 ± 0.01	103.0%	5.6%	5.4%	3.5%
Copper	15.5 ± 0.63	14.2 ± 0.69	90.6%	3.4%	3.7%	2.1%
Iron	347 ± 20	325 ± 13	95.4%	3.9%	4.1%	2.4%
lead	0.395 ± 0.05	0.140 ± 0.01	36.5%	3.0%	8.1%	1.8%
Mercury	0.38 ± 0.060	0.37 ± 0.020	93.8%	7.5%	8.0%	4.7%
Zinc	51.3 ± 3.1	48.1 ± 1.6	98.8%	9.5%	9.6%	5.9%

n – number of replicates, **Std dev** – standard deviation, **%RSD** – percentage relative standard deviation, **95%C.I.** – 95 percent confidence interval.

Table 2.7: Percentage recoveries and statistical summary of trace elements in bovine liver and tomato leaves standard reference materials

Trace elements	SRM certified values	Measured SRM values	Mean % recovery	Std dev	%RSD	95% C.I.
Bovine Liver Standard Reference Material (SRM 1557c, NIST, mg kg^{-1}), n = 10						
Arsenic	0.0196 ± 0.0014	<LOQ	-	-	-	-
Cadmium	0.097 ± 0.0014	0.102 ± 0.0070	105.5%	7.2%	6.8%	4.5%
Copper	275.2 ± 4.6	256.3 ± 18.9	93.1%	6.9%	7.4%	4.3%
Iron	197.94 ± 0.65	205.36 ± 18.01	103.9%	9.2%	8.8%	5.7%
Lead	0.0628 ± 0.001	0.0583 ± 0.0065	93.0%	10.3%	11.03%	6.4%
Mercury	0.00536 ± 0.00017	0.00536 ± 0.00053	99.9%	9.9%	9.9%	6.1%
Zinc	181.1 ± 1.0	182.4 ± 14.7	100.7%	8.1%	8.0%	5.0%
Tomato leaves Standard Reference Material (SRM 1573a, EVISA, mg kg^{-1}) n = 6						
Arsenic	0.112 ± 0.004	<LOQ	-	-	-	-
Cadmium	1.52 ± 0.04	1.39 ± 0.10	91.1%	6.8%	7.4%	5.4%
Copper	4.70 ± 0.14	4.62 ± 0.21	98.3%	4.5%	4.5%	3.6%
Iron	368 ± 7	345 ± 17	93.6%	4.8%	5.1%	3.8%
Lead	NP	0.38 ± 0.19	-	-	-	-
Mercury	0.034 ± 0.004	ND	-	-	-	-
Zinc	30.9 ± .7	29.3 ± 1.5	94.9%	4.7%	5.0%	3.8%

NP- certified value not provided, **ND**- not detected, **n** – number of replicates, **Std dev** – standard deviation, **%RSD** – percentage relative standard deviation, **95%C.I.** – 95 percent confidence interval.

2.4.4 DETERMINATION OF LOD AND LOQ IN SEDIMENT AND BIOTA

Instrument limits of detection (LOD) for sediments and biota were determined (Table 2.8) as described by the USEPA (USEPA 2005). Although LOD was calculated in a similar manner to seawater (three times the standard deviation of the blanks), the LOQ was determined slightly differently. The LOQ was calculated by taking into account the weight of the sample that was digested and the dilution factors for the analysis by ICP-MS.

The statistically-derived LOD's were lower than the actual solution concentrations that could routinely be achieved in natural samples (with the exception of zinc in biota). Therefore, higher practical LOD's were estimated and applied. These detection limits were estimated by checking the quantitative recoveries of low concentration elements in certified reference materials, percentage recoveries of low concentration elements in spiked analyses, percentage differences in duplicate samples, and the reproducibility of the reading of the lowest standard used in analytical runs. The effective LOQ's were obtained using the estimated and applied practical LOQ and the dilution factor.

Table 2.8: Detection and quantitation limits for ICP-MS and AAS for trace elements in sediment

Analyte	Calculated ICP-MS LOD for sediment (µg L^{-1})	Estimated and applied practical LOQ for sediment (µg L^{-1})	Effective LOQ for sediment (µg g^{-1})
Arsenic	0.480	1.00	0.42
Cadmium	0.002	0.05	0.02
Copper	0.070	0.1	0.04
Iron*	60.00	80.00	33.6
Lead	0.050	0.15	0.04
Mercury	0.005	0.20	0.08
Zinc	1.080	2.50	1.05

LOD – limit of detection, **LOQ**- limit of quantification. *****Sediment iron analysis by AAS instrument

Table 2.9: Detection and quantitation limits for ICP-MS analysis of trace metals in biota

Analyte	Calculated ICP-MS detection limits (µg L^{-1})	Estimated and applied practical quantitation limit (µg L^{-1})	Effective quantitation limit for biota* (µg g dry wt^{-1})	Effective quantitation limit for algae (µg g dry wt^{-1})	Effective quantitation limit for fish tissue (µg g dry wt^{-1})
Arsenic	0.200	1.00	0.500	1.000	0.20
Cadmium	0.005	0.05	0.025	0.500	0.01
Copper	0.040	0.10	0.050	0.025	0.02
Iron	1.950	10.00	5.000	0.050	2.00
Lead	0.020	0.10	0.050	5.000	0.02
Mercury	0.040	0.20	0.100	0.050	0.04
Zinc	1.400	1.00	0.500	0.100	0.20

*"biota"- fish tissue, whole tissue of green mussel, crab muscle and whole body marine worm

2.5 SAMPLE ANALYSES

2.5.1 TRACE ELEMENT ANALYSIS BY ICP-MS

Trace element analysis of the seawater extract and diluted acid digest (sediment and biota) for arsenic (^{75}As), cadmium (^{111}Cd), copper (^{63}Cu), iron (^{57}Fe), mercury (^{201}Hg), lead (sum of ^{206} Pb, ^{207}Pb and ^{208}Pb) and zinc (^{66}Zn) was carried out using an Agilent 7500cx ICP-MS instrument. The sole exception was iron in sediment, which was analysed by AAS (see Section 2.5.2). The working range of the ICP-MS instrument was from 0 to 1000 µg L^{-1}. The instrument was calibrated using trace element standards of 0, 0.1, 1, 10, 100 and 1000 µg L^{-1}, with the acceptable calibration curve R^2 value being >0.999. The standards were prepared by volumetric dilution of a 10,000 µg L^{-1} mixed metal stock solution (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and a single element mercury stock standard (10,000 µg L^{-1}), with the same acid mixture used for the dilution of sediments and biota, to match the matrix of the sample and the standards. Concentrations of mixed elements were sometimes different from the concentrations of mercury in the same standard to prevent contaminating the apparatus with high levels of mercury, and thus ensure good and reliable washout in a reasonable timeframe. The concentrations of mercury and concentrations of mixed elements in the 0, 0.1 and 1 µg L^{-1} standards were the same, while the 10, 100 and 1000 µg L^{-1} mixed element standards contained 2, 5 and 10 µg L^{-1} of mercury respectively.

Table 2.10: Operating parameters for the ICP-MS

RF power (W)	1560
Sample depth (mm)	9.2
Carrier gas (L min^{-1})	0.8
Make up gas (L min^{-1})	0.2
Peristaltic pump (RPS)	0.1
Spray chamber (°C)	2
Helium flow (mL min^{-1})	4.5

A standard reference material of water (SRM 1643e) was run daily to check the calibration. Standards were analysed every 20 samples at 0, 2 and 20 µg L^{-1} to check for instrument drift, and an internal standard ^{103}Rh (rhodium) was added on-line

to account for drift. The check standards of mixed elements at 0, 2 and 20 μg L^{-1} contained 0, 0.2 and 2 μg L^{-1} of mercury respectively. The ICP-MS was run in collision mode using He to remove polyatomic interferences formed in the plasma and was tuned daily using a tuning solution of 1 μg L^{-1} cerium (Ce), cobalt (Co), yttrium (Y), lithium (Li) and thallium (Tl). The ICP-MS instrument operating parameters are provided in Table 2.10.

2.5.2 IRON ANALYSIS BY AAS

Iron was analysed by flame AAS (Varian SpectrAAS220FS), using an air/acetylene flame, and absorbance with concentration as the calibration mode. The wavelength was 248.3 nm with a slit width of 0.2 nm. Six calibration standards (0, 10, 25, 50 75 and 100 mg L^{-1}) were freshly prepared by volumetric dilution of a single element iron stock solution (1000 mg L^{-1}) with 2% nitric acid. The R^2 of the calibration graphs was always greater than 0.9948 ($0.9948 < R^2 > 0.9981$). The instrument was always re-calibrated after 30 samples. Samples analysed by AAS were diluted by a factor of 21 using 2% HNO_3, and those which gave results outside of the calibration range were diluted by a factor of 51 and reanalysed.

CHAPTER 3

THE SEA-FILL OF THILAFUSHI ISLAND OF THE MALDIVES: ENVIRONMENTAL CHARACTERISATION AND RISK ASSESSMENT FOR CONSUMPTION OF LOCALLY-SOURCED SEAFOOD

3.1 INTRODUCTION

Sea-fills and coastal landfill activities are potential sources of trace elements (Denton et al. 2001; Jones 2010; Kjeldsen et al. 2002), and monitoring of trace element concentrations in various matrices around sea-fills is important to determine the toxic effects that these elements may have on the aquatic biota and seafood consumers. Seafood consumption has been reported as a significant exposure route for humans to toxic trace elements including arsenic, mercury, cadmium and lead (Agusa et al. 2007; Borak & Hosgood 2007; Falconer et al. 1983; Han et al. 1998; Meador et

al. 2004; Olmedo et al. 2013; Phillips et al. 1982; Wang et al. 2013; Ysart et al. 2000). Limits for trace element levels in food standards are set to minimise health impacts due to the intake of contaminated foods (WHO 2000,2007,2010a,b).

Although there has been extensive research monitoring trace elements in the environment in various parts of the world, few studies have been carried out in the developing world, where most of the contamination occurs (Li et al. 2009; Pacyna & Pacyna 2001). One potentially contaminated site in the Maldives is an artificial island (Thilafushi Island) that was created in a lagoon through accumulation of solid wastes. Leaching of trace elements from Thilafushi Island into the lagoon and the surrounding marine environment could be a potential health hazard as local people consume fish and other seafood harvested from the lagoon and the surrounding sea.

Fish is an important source of protein and a significant component of diets in some parts of the world (Agusa et al. 2007; Kawarazuka & Béné 2011; Olmedo et al. 2013; Wang et al. 2013; Yilmaz 2003) including Maldives. Maldivians depend heavily on fish, with this source contributing approximately 88% of total protein intake (Golder et al. 2001). Maldivians are the top consumers of fish in the world, consuming an average of 160.3 kg of fish per capita per year (Harrison & Pearce 2000), making them more reliant on fish than any other surveyed countries (Kawarazuka & Béné 2011).

Local people of the Maldives consume both deep water and reef fish. Some commonly consumed reef fish include snapper (Lutjanidae), grouper (Serranidae), jack (Carangidae), bigeye scad (*Selar crumenophthalamus*), surgeon fish (Acanthuridae), wrasse (Labridae), mullet (Mugilidae), goatfish (Mullidae), as well as a variety of different species of “bait” fish (Adam 1995; Kawarazuka & Béné 2011). Other reef organisms consumed, but to a lesser extent, include lobster, octopus, crab, squid and shellfish. Commonly consumed deep water fish include different species of tuna and swordfish (Kawarazuka & Béné 2011). Fish organs including liver and gonad are considered to be delicacies in the Maldives (Bluepeace 2008).

Data on trace element concentrations in the Maldivian food chain are nonexistent. However, baseline data are available on trace element levels in marine sediments from Thilafushi Island via an environment impact assessment (EIA) report

prepared for a solid waste management facility at Thilafushi Island (CDE Consulting 2011). Therefore, a multifaceted study was designed and carried out to address current levels of trace elements associated with the sea fill, and to measure for the first time, levels of trace elements in seafood. Seawater, marine sediment and selected marine biota were collected around the sea-fill of Thilafushi Island and a reference site (Huruelhi Island) in the Maldives for trace element analysis. The trace elements of interest in this study were arsenic, cadmium, copper, iron, mercury, lead and zinc, because they are known to be present in the solid waste fill leachates and can pose significant threat to the surrounding environment and humans (Jones 2010; Kjeldsen et al. 2002; Maata & Singh 2008). This study was particularly focused on arsenic, cadmium, mercury and lead because they are non-essential elements, known to cause toxic effects at low concentrations.

The specific objectives of the present study were to:

- Characterise trace element concentrations in seawater and marine sediment from the vicinity of the Thilafushi Island sea-fill site, and a reference site (Huruelhi Island)
- Produce baseline data for trace elements in marine biota from the Maldives
- Perform a risk assessment for consumption of fish harvested from the vicinity of Thilafushi Island

3.2 MATERIALS AND METHODS

The laboratory experimental methods used to extract trace elements from seawater, marine sediments and biota, and their preparation for chemical analysis, are provided in Chapter 2.

3.2.1 STUDY SITE

The Maldives is an archipelago of 1,190 coral islands, divided into 26 atolls for administrative purposes. These islands are spread over an area of approximately 750 km from north to south and 120 km from east to west (Peterson 2013; van den

Akker & Saleem 2007).

Thilafushi Island (Figure 3.1) was developed during the 1990's to solve the waste disposal issues of the capital city, Male', and the reclamation work with MSW began in 1992 (Goverment of Maldives 2008; Government of Maldives 2010; Peterson 2013). Thilafushi Island is now the main waste disposal site for Male' and surrounding islands, including several tourist resorts (Goverment of Maldives 2008; Government of Maldives 2010; Peterson 2013; UNEP 2005a). It is estimated that more than 860 tons of wastes are generated every day around the Maldives (Peterson 2013), of which more than 400 tons per day are brought to Thilafushi Island for disposal (IFC 2009).

Figure 3.1: Thilafushi Island and surrounding lagoon at the start of this project (2011). Map adapted from Google Earth

During the early years of waste disposal operations, cells with size of approximately 1060 m^3 were dug on the fringing coral reef and sand obtained from the excavation was used to construct walled enclosures around the internal perimeter of the cells. Stockpiled waste was used to fill the pits, which were topped off with a layer of construction debris, and then uniformly leveled with excavated white sand (personal observation). The reclamation process did not involve any lining of the

seabed or creation of silt curtains adjacent to the MSW fill site to mitigate leaching of materials into the surrounding environment.

The Maldives government started leasing the reclaimed land for entrepreneurs interested in acquiring land for industrial purposes in 1997 (CDE Consulting 2011; Peterson 2013). The current major industrial activities on the island are boat building, cement packing, methane gas bottling and various large scale warehousing (CDE Consulting 2011). On-site open air burning and burning of stockpiled solid waste using incinerators has been introduced recently (Peterson 2013).

3.2.1.1 PHYSICAL ENVIRONMENTAL CONDITIONS

The seawater in the inner lagoon of Thilafushi showed visible signs of pollution at the time of sample collection. The inner lagoon was turbid and dirty green in colour unlike other lagoons in the Maldives which are typically clear and turquoise in colour. This discolouration could be due to leachate directly running off from the adjacent waste dump into the lagoon. The reef areas directly open to the deep ocean were more clear (similar to other lagoons in the Maldives), most likely due to the dilution of leachate by the open sea. The inner lagoon (Figure 3.1) is a semi-enclosed system covered by the reclaimed land, and the surrounding shallow water reef flats can restrict direct dilution of contaminants seeping into the inner lagoon.

The inner lagoon of Thilafushi Island is a low energy environment (CDE Consulting 2011). The sea current at both high and low tides in the inner lagoon flows mainly from south to north with an average speed of 0.02 - 0.3 m/s (CDE Consulting 2011). The highest observed sea current was on the reef flat immediately after the breaker zone near the sand reclaimed area (Figure 3.2A) at an average speed of 1.15 m/s (CDE Consulting 2011). The reference site is an uninhabited small island (Huruelhi Island) in Ari Atoll, south of Male' Atoll, with a similar natural geological and physicochemical environment to Thilafushi Island.

Seawater and marine sediments were collected along four transect lines (T1-3 on Thilafushi Island, T4 on Huruelhi Island). Transect line T1 passed through the inner lagoon starting from approximately 2 m from the shoreline (Figure 3.2A). The surrounding land was the first MSW-reclaimed area. The adjacent areas (shown in Figure 3.2A) are still being used for dumping wastes and open burning of MSW

(observed during field sample collection). The inner lagoon is also extensively used by ships and boats as a sheltered location for anchorage for various purposes including repair and maintenance works. Wastes from the repair works are also continuously being released directly into the inner lagoon (observed during the field sampling). Loading and unloading of commercial goods from boats and ships was being carried out adjacent to the land of the inner lagoon at the time of sampling.

Figure 3.2: Transect lines and sample collection points on Thilafushi Island (A). Transect lines and sample collection points on Huruelhi Island (B).
Map adapted from Google Earth

Transect line T2 was adjacent to the sand-only reclaimed area (Figure 3.2A). The sand used for reclamation at the T2 site was dredged from the west end of the

inner lagoon. Therefore, transect line T2 was not directly connected to the MSW disposal sites. Transect line T3 passed through the reef towards the open sea at the boat yard (Figure 3.2A). The adjacent land to T3 was part of the initial reclaimed area. However, there are still some waste disposal sites (towards the west) at a close proximity to transect line T3. Wastes from boat repair and maintenance work are directly released into the adjacent sea. Transect line T4 (Huruelhi Island; Figure 3.2B) was relatively free of any human activities, except snorkeling by tourists from nearby resorts and some recreational reef fishing activities by local islanders.

3.2.1.2 SOURCES OF TRACE ELEMENTS IN MSW

The solid wastes disposed of at Thilafushi Island include domestic and industrial waste of organic and inorganic nature, and includes a wide range of wastes from small tins to whole cars (Khaleel & Saeed 1997). The solid wastes are mixed with all types of metals, e-waste and other potentially hazardous chemicals including hospital wastes, lead acid batteries, ceramics, light bulbs, house dust, paint chips, lead foils, used motor oils, plastics, inks and glass (UNEP 2005b). These wastes can contribute trace elements such as iron, copper, arsenic, cadmium, mercury, and lead to the surrounding environment (Ettler et al. 2008; Whittle & Dyson 2002).

3.2.2 SAMPLE COLLECTION AND PREPARTION

All samples for this study were collected in September, 2012. Seawater and marine sediments were collected from the same GPS locations along the three transect lines T1, T2, and T3 around Thilafushi Island (Figure 3.2A) and one transect line (T4) in the reference site (Huruelhi Island) (Figure 3.2B). The distances from the shoreline to each sampling site within each transect were 2 m, 20 m, 80 m, 160 m and 400 m. Seawater was collected before sediment samples to avoid any contamination in the seawater due to sediment collection work.

3.2.2.1 ENVIRONMENT CHARACTERISATION SAMPLES

3.2.2.1.1 SEAWATER

Surface seawater samples (50 mL) were collected as one-off samples at low tide. One field blank (50 mL Milli-Q water) for each transect line was included.

Triplicate seawater samples (one sample for pH recording, two for trace element analysis) were collected by an acid-cleaned plastic jug tied on an untreated wooden pole of 1.5 meters long. The GPS coordinates of the sampling sites were recorded (Appendix A1). The pH values of the seawater samples were recorded immediately upon collection (Appendix A1). The seawater samples for trace element analysis were placed on ice until transported to the laboratory (Maldives Food and Drug Authority - MFDA), where they were kept at 4°C until couriered (within a week of collection) to New Zealand on ice. All seawater samples were acidified to pH ≤ 2, with ultrapure nitric acid and stored at 4°C upon arrival at the University of Canterbury, New Zealand.

3.2.2.1.2 MARINE SEDIMENT

Marine sediments were collected by divers using acid-cleaned plastic jars. The sediment samples were placed on ice until transported to the laboratory (MFDA) and dried in an oven at 30°C in aluminum trays until constant weight (7 - 10 days). Other steps involved in sample preparation, digestion and analysis were described in Chapter 2.

3.2.2.2 MARINE BIOTA

Four different groups of biota were collected around the sea-fill and reference sites. They were green algae, a marine worm (*Sipunculus indicus*), a shellfish (penguin wing oysters, *Pteria penguin*), and two species of fish (parrotfish, *Scarus ventula*; red mullet or Indian goatfish, *Parupeneus indicus*). An attempt was made to collect crabs, but only one animal was able to be obtained at the sea-fill site.

The selected fish species have a restricted home range, are endemic to Maldives coastal waters, and easy to catch. They are suitable biomonitoring species as their accumulated trace element levels are likely to reflect the area from which they are caught (Phillips 1977; Rainbow 1995). Additionally, these species of fish are consumed by Maldivians, and trace element data are therefore relevant for risk assessment for consumption of fish. Red mullet and parrotfish (Figures 3.3A and 3.3B respectively) were collected from the study sites by set nets. Fish were euthanised according to the University of Canterbury Animal Ethics approval, by immersing them into a solution prepared in a clean bucket with 2-phenoxyethanol and seawater

(approximately 0.5 to 1 mL of 2-phenoxyethanol per litre of seawater, ~0.3-0.6 mg L^{-1}). The spinal cord was then severed to ensure euthanasia, fish were rinsed with fresh seawater, and placed in plastic snap-lock bags on ice until transported to the laboratory (Figure 3.3).

A sentinel bivalve species was selected as these are suspension feeders that take trace elements directly from the dissolved phase and also suspended particles during filter feeding (Rainbow 1995). Tissue burdens of bivalves are known to reflect environmental contamination levels (Amiard-Triquet et al. 1992b; Kennedy 1986; Peake et al. 2006; Rainbow 1995; Reinfelder et al. 1998). Since shellfish were not readily available in the accessible area of Thilafushi sea-fill site, penguin wing oysters (*Pteria penguin*) were collected at the far end of Thilafulhu lagoon, as it was the only species of bivalve that was found in common to the sea-fill and the reference site. Locating any site with shellfish was difficult, possibly due to the contaminated and highly disturbed environment. The penguin wing oysters (Figure 3.4C) were collected by divers from black corals.

A marine worm was selected as these organisms take in trace elements predominantly from ingestion of sediment-bound materials (Fauchald & Jumars 1979; Meador et al. 2004; Rainbow et al. 2006a,b), and trace element burdens in marine worms can directly correlate with the concentrations in the sediment (Meador et al. 2004; Phillips 1990). Peanut worms (Sipunculidae) were the only species that were reasonably available, common to both the sampling sites, and easy to collect. Marine worms (Figure 3.4B) were collected by divers by finger raking in the lagoon sand. These worms were sand filled; therefore, the sand was squeezed out immediately after capture, before wet weights were recorded.

Green algae were selected as a primary producer. The principle mechanism of nutrient and trace element uptake by the algae is via physicochemical adsorption to the surface (Macfie & Welbourn 2000; Robinson et al. 2006). Green algae were collected by scraping from rocky concrete-like hard materials at the shore line of Thilafushi Island (Figure 3.4A). The green algae were scarce at Huruelhi Island and were only present on the dead coral pieces in the shallow area near the shoreline. The wet weights of the algae samples were recorded, and algae were then oven dried at 50°C until constant weight (5 - 7 days) at MFDA laboratory, and kept at -20°C in acid

cleaned plastic vials until transported to the University of Canterbury. There the samples were freeze-dried to remove any moisture that may have been absorbed in the handling process.

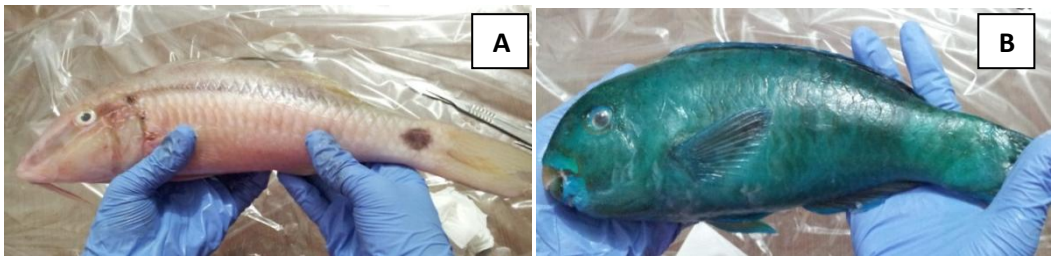


Figure 3.3: Goatfish (*Parupeneus indicus*) (A), and Parrotfish (*Scarus ventula*) (B)

The whole body weights of shellfish and their shell lengths were recorded before all soft tissues were dissected and collected for analysis. The fork lengths and whole body weights of fish were also recorded before dissection. Fish muscle, liver, gonad and kidney were collected. The wet weights of all samples were recorded and tissues were stored in acid-cleaned plastic vials at -20^{o}C in the MFDA laboratory, and transported to New Zealand on dry ice. These samples were kept at -20^{o}C in a containment laboratory at the University of Canterbury until analysis. Detailed description of digestion and analysis of biota is provided in Chapter 2. Appendix A2 provides an overview of the biota samples and their physical dimensions.

Figure 3.4: Green algae (A), *Sipunculus indicus* (B), *Pteria penguin* (C)

3.2.2.3 METAL POLLUTION INDEX (MPI)

The metal pollution index (MPI) was calculated and used to compare trace element contents at the sea-fill and reference site using the trace element

concentrations in seawater and marine sediment. The MPI values were calculated using Equation 3.1 (Usero et al. 2005)

$$MPI = (Cf_1 \times Cf_2 \dots Cf_n)^{1/n} \qquad \textit{Equation 3.1}$$

where Cf is the concentration of the trace element (dry weight), and n is the number of trace elements analysed.

3.2.2.4 RISK ASSESMENT FOR CONSUMPTION OF SEAFOOD

Exposure scenarios were created as anthropometric data for different age groups of Maldivians are not readily available, and no diet survey has been carried out for any food, including fish. In this regard, the weekly intake of contaminants per kilogram of body weight was calculated for a male adult (65 kg), female adult of child-bearing age (49 kg), child (30 kg) and toddler (13 kg) (Golder et al. 2001). The three different fish consumption rates chosen were the FAO value of 420 g per day (FAO 1999), a diet based on reports that Maldivian women and children consume fish (mainly tuna) at a rate of 80 g per day (Golder et al. 2001) and an intermediate consumption rate of 250 g of fish per day. The consumption rate chosen for toddlers was 80 g per day, assuming that toddlers would not consume as much fish as adults and children. The trace element concentrations of red mullet from Thilafushi Island were used in the risk assessment calculations, as this fish species contained the highest concentrations of trace elements in the muscle of the two fish species analysed.

Trace element exposures were estimated for the toxic elements arsenic, cadmium, mercury and lead. The World Health Organization (WHO) and the United States Food and Drug Administration (USFDA) assume that less than 10% of the total arsenic in fish and shellfish is present as inorganic arsenic (WHO 2010a). These values are consistent with the literature values for proportion of total arsenic (Gagnon et al. 2004). For example, the inorganic arsenic content in fish and shellfish ranged from 2% to 7% of total arsenic (Muñoz et al. 2000), and Gagnon et al. (2004) reported a mean of 8.2% inorganic arsenic in shellfish. For total mercury, 100% was assumed to be methylmercury (MeHg) as methylmercury is the predominant form of mercury in fish (93-99.9%) (Hight & Cheng 2006; Olmedo et al. 2013).

The trace element exposure doses were calculated using U.S. Environmental Protection Agency (USEPA) guidelines that were used to generate advisories for risk assessment and fish consumption limits (USEPA 2000). The weekly intake of the trace elements was calculated using Equation 3.2, where EWI is the estimated weekly intake of trace elements (µg/kg BW/week), MI is the fish consumption rate per person per week (g/person/week), MC is the concentration of trace elements in the edible tissue (µg g wet wt^{-1}), BW is the body weight of the consumer (kg).

$$EWI = \frac{MI \times MC}{BW} \qquad \textit{Equation 3.2}$$

Scenario 1 was based on two different contaminant concentrations (mean and 95^{th} percentile) of fish muscle by all four body weight (BW) categories for the three fish consumption rates. Scenario 2 was based on the consumption of fish muscle and fish organs (liver and gonad), because fish organs are considered as delicacies in the Maldives. It was assumed that an average Maldivian consumes fish organs, mainly gonad and liver, at least once a week along with fish muscle. The mean concentrations of muscle and organs were considered at the medium fish consumption rate for the male, female and the child, but the lowest consumption rate for the toddler, assuming that toddlers do not consume as much fish as the other BW groups. The average wet weight of liver and gonad were used in the trace element intake calculations.

Scenario 3 was based on the consumption of fish muscle, fish organs (liver and gonad), and shellfish, although shellfish is not a very commonly consumed seafood in the Maldives. Inclusion of shellfish would represent a worst case scenario for contaminant intake levels. Scenario 3 assumed at least one shellfish (average size of shellfish in this study) is consumed per week along with the fish muscle and organs (as in Scenario 2) by each category of consumer with the exception of toddlers. The mean concentrations of trace elements in all species/tissues were used for the EWI calculations with the medium fish consumption rate.

3.2.2.5 STATISTICAL ANALYSIS

All statistical analyses were carried out in R© (Version 2.15.3). For analysis of

trace elements, statistics were only performed for sample sets where more than 50% of the samples had values above the limit of quantification (LOQ). In conditions where more than 50% of the samples were above the LOQ, the remaining samples below LOQ were given a value of half the LOQ. All duplicate measurements were averaged before inclusion in the statistical analysis.

All data were checked for normality by plotting probability plots. Where necessary, data were log transformed to meet assumptions of normality before analysis. Significant differences ($p < 0.05$) at the 95% confidence level for trace element concentrations between the sites, between the transect lines (T1 to T4) and with distance from shoreline (only for the sea-fills site, T1, T2 and T3) were determined by using two-way ANOVA (factors being transect line and distance) followed by Tukeys HSD test. Significant differences between the biota species with respect to site, differences between organs within the same fish, and difference between species within the same site, were assessed by one-way ANOVA followed by Tukeys HSD test. Pearson's correlation tests were also used to analyse relationships between trace elements within and between the environmental matrices.

3.3 RESULTS

3.3.1 ANALYTICAL PERFORMANCE

Details of percentage recoveries in the QA (quality assurance) samples are provided in Appendix A3 for each of the environmental matrix types.

3.3.1.1 SEAWATER

Percentage recoveries of cadmium, copper, iron, lead and zinc in certified reference seawater and trace element-spiked Milli-Q water, real seawater, artificial seawater and certified reference seawater (NASS-6) ranged from 83 to 122% (Table A3.1 in Appendix A3). The percentage recovery for iron ranged from 83 to 97% for the trace element-spiked samples. Quality assurance (QA) water samples were extracted in duplicate.

3.3.1.2 MARINE SEDIMENTS

The percentage recoveries of trace elements in marine sediment standard reference materials (SRM-2702-NIST) ranged from 92 to 115%.

3.3.1.3 BIOTA

The percentage recoveries of all elements in the standard reference mussel and fish protein (DORM-3) were over 90%, with the exception of lead (34.9%) for DORM-3. The mean percentage recoveries of all elements in certified reference bovine liver were over 90.7% with the exception of lead (88.7%). Arsenic and mercury were below the limit of quantification. Mean percentage recovery of cadmium, copper, iron and zinc were over 98.5% in tomato leaves (Table A3.2 in Appendix A3). Arsenic and mercury concentrations in tomato leaves were below their respective limits of quantification, and a certified value for lead was not provided.

3.3.2 ENVIRONMENTAL CHARACTERISATION

The mean and range of trace element concentrations in seawater and marine sediments from each of the transect lines is provided in Tables 3.1 and 3.2. Guideline values for seawater and marine sediments (Australian and New Zealand Environment and Conservation Council; ANZECC), metal pollution index (MPI) values derived for each transect line for each of the matrices, and comparisons of trace elements with similar studies are presented in Tables 3.1 and 3.2 for seawater and marine sediments respectively.

3.3.2.1 TRACE ELEMENT CONCENTRATIONS IN SEAWATER

The pH values of the seawater samples ranged from 7.5 to 8.2 and were not significantly different between the two sampling sites. The concentration of trace elements in the seawater followed the order: iron > zinc > copper > lead > cadmium for both sites. The variation of trace element concentrations in seawater along the transect lines is presented in Figure 3.5. Concentrations of all trace elements were significantly higher in the seawater at the sea-fill site than at the reference site. In general, the concentrations of trace elements at T1 and T3 were significantly higher than those of T2 and T4, with the exception of cadmium, where concentrations were

not significantly different between T1, T2 and T3. However, the cadmium concentrations at T1 were significantly higher than those of T4 (reference site). Concentrations of all trace elements at the transect line T2 (sand-reclaimed site) were not significantly different from those of the transect line T4.

There were no significant differences with respect to distance from shoreline for any trace element at the sea-fill site when the three transect lines (T1, T2 and T3) were combined. However, T3 showed a clear drop-off for all trace elements with distance from shoreline. Pearson's correlation analysis of trace elements in seawater of Thilafushi Island indicated that all trace elements were significantly and positively correlated to each other, while there were no significant correlations between any elements in the seawater from the reference site (Table A5.1 in Appendix A5). The MPI values for seawater (Table 3.1) indicated that trace element contamination was highest at the site of transect line T3 (adjacent to the boat yard), followed by T1 (inner lagoon) and T2 (sand reclaimed site). The MPI value was lowest at the reference site (T4).

3.3.2.2 TRACE ELEMENT CONCENTRATIONS IN MARINE SEDIMENTS

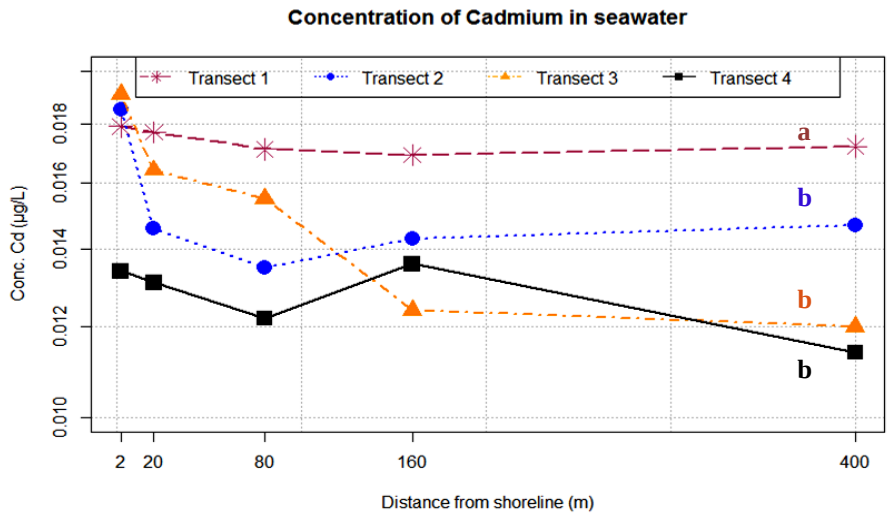
Mercury was <LOQ for all the marine sediment samples. Figure 3.6 presents patterns of individual trace elements in marine sediments as a function of distance from the shoreline. Arsenic and cadmium concentrations at T1 were significantly higher than those of T2, T3 and T4. However, overall comparisons of Thilafushi sediment samples to Huruelhi samples indicated that there were no significant differences for arsenic and cadmium levels between the two sites. Transect lines T1 and T3 contained significantly higher concentrations of copper, iron, lead and zinc than T2 and T4, while T1 was significantly elevated in these elements relative to T3.

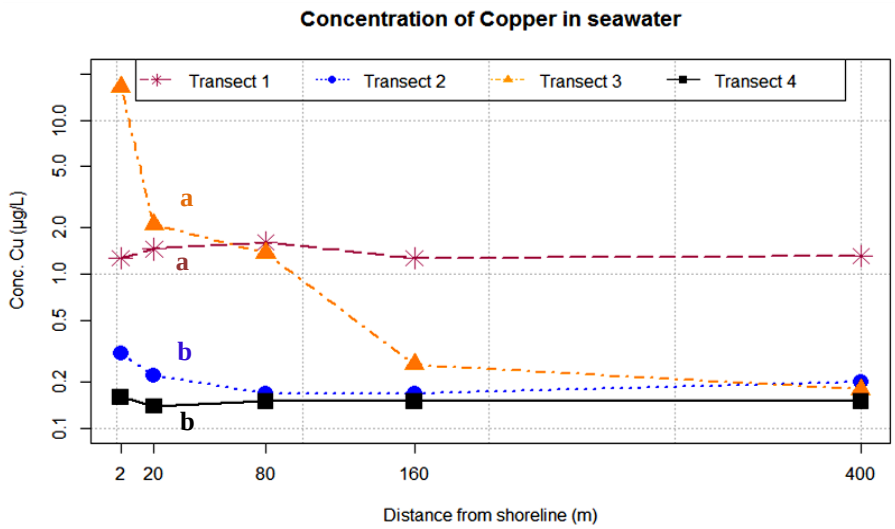
In general, all the trace elements were significantly higher at transect line T1 (in the inner lagoon) and T3 (adjacent to the boat yard) than at T2 (adjacent to the sand-only reclaimed area at the sea-fill, away from the MSW dump sites) and T4 (reference site). There were no significant differences between T2 and T4 for any trace element in the sediments. No trace element varied significantly with distance at the sea-fill site when the three transect lines were combined (T1, T2 and T3), but

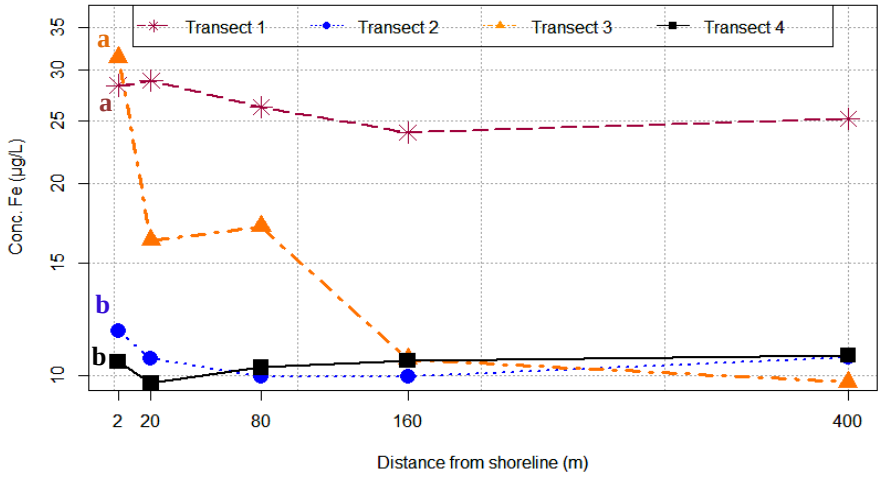
individually the T1 and T3 transects showed a clear decrease with distance from the shoreline.

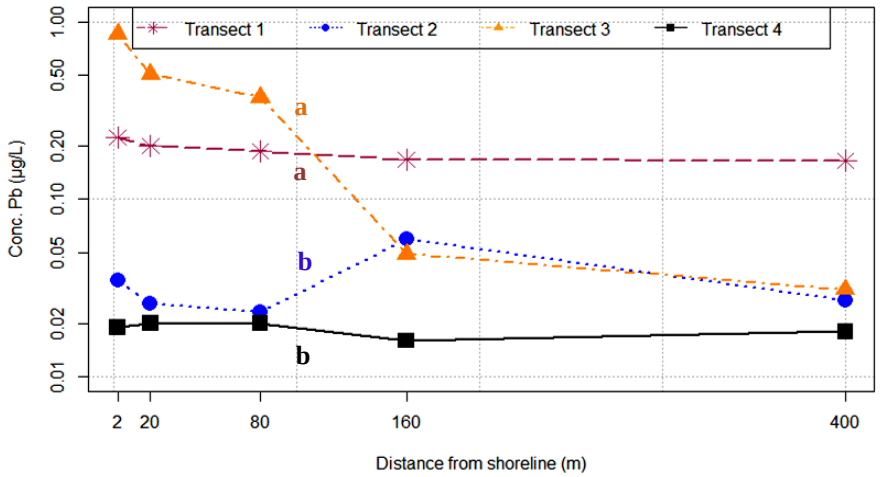
Correlation analysis of trace elements in marine sediments of Thilafushi Island indicated that all trace elements were significantly and positively correlated to each other, while only copper and cadmium were significantly positively correlated in the sediments at the reference site (Table A5.2, Appendix A5). There were no significant correlations between seawater trace elements and marine sediment trace elements (Table A5.3, Appendix A5). The highest MPI value for the sediments (Table 3.2) was derived for transect line T1, followed by T3 and T4 (the reference site). The lowest MPI value was obtained for T2.

Particle size and total organic carbon (TOC) contents of the sediments were not determined due to restrictions on removing the samples from the PC2 laboratory. Therefore, the particle size and TOC data from the EIA report of Thilafushi (CDE Consulting 2011) were used in the discussion.


Concentration of Cadmium in seawater
Transect 1
Transect 2
Transect 3
Transect 4
Conc. Cd (µg/L)
0.010
0.012
0.014
0.016
0.018
a
b
b
b
2
20
80
160
400
Distance from shoreline (m)


Concentration of Copper in seawater
Transect 1
Transect 2
Transect 3
Transect 4
Conc. Cu (µg/L)
0.1
0.2
0.5
1.0
2.0
5.0
10.0
a
a
b
b
2
20
80
160
400
Distance from shoreline (m)


Concentration of Iron in seawater
Transect 1
Transect 2
Transect 3
Transect 4
Conc. Fe (µg/L)
10
15
20
25
30
35
a
a
b
b
2
20
80
160
400
Distance from shoreline (m)


Concentration of Lead in seawater
Transect 1
Transect 2
Transect 3
Transect 4
Conc. Pb (µg/L)
0.01
0.02
0.05
0.10
0.20
0.50
1.00
a
a
b
b
2
20
80
160
400
Distance from shoreline (m)

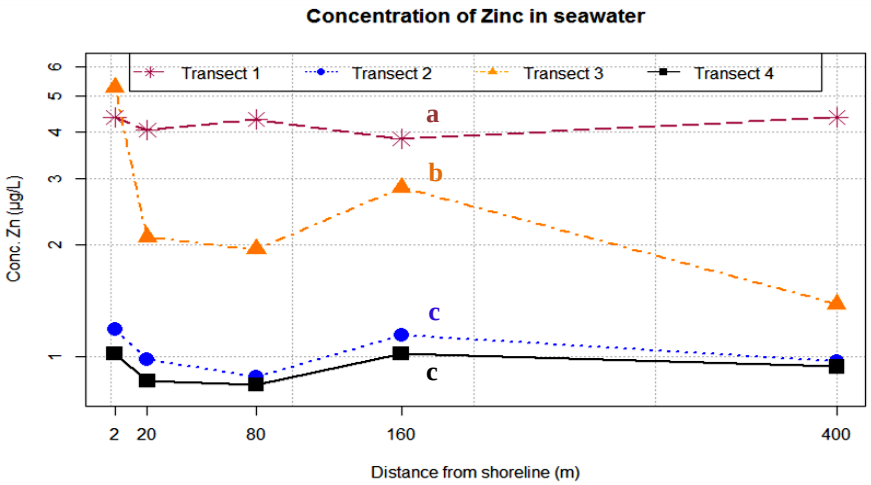


Figure 3.5: Trace element concentrations in seawater along the four transect lines. Transect lines sharing letters are not significantly different.

Table 3.1: Mean and range of trace element concentrations in seawater, MPI values for each transect line, ANZECC guideline values, and comparisons of trace element concentrations in seawater with other similar studies

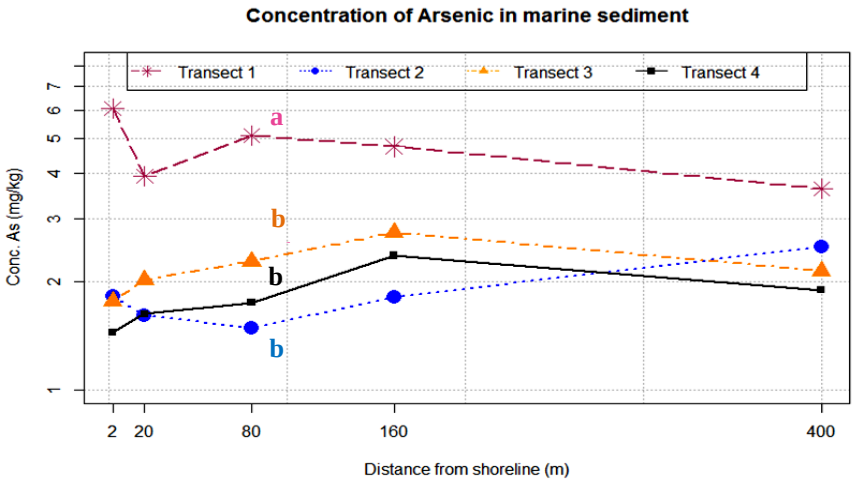
Sample / location	Fe	Cu	Zn	Cd	Pb	MPI
Seawater (µg L^{-1})						
T1	26.56 (24 - 29)	**1.39** (**1 - 2**)	4.20 (3.84 - 4.38)	0.017 (0.017 - 0.018)	0.19 (0.17 - 0.22)	0.87
T2	10.60 (10 - 12)	0.21 (0.17- **0.31**)	1.03 (0.88 - 1.18)	0.015 (0.014 - 0.019)	0.034 (0.023 - 0.060)	0.26
T3	17.04 (10 - 31)	**4.06** (0.18 - **16.37**)	2.70 (1.38 - 5.26)	0.015 (0.012 - 0.019)	0.36 (0.03 - 0.85)	1.00
T4 –reference site	10.38 (10 - 11)	0.15 (0.14 - 0.16)	0.94 (0.84 - 1.02)	0.013 (0.011 - 0.014)	0.019 (0.016 - 0.020)	0.20
ANZECC trigger values for marine water (µg L^{-1}) for level of protection of marine species (% species)						
99% protection	**	0.30	7.00	0.70	2.20	
95% protection	**	1.30	15.00	5.50	4.40	
90% protection	**	3.00	23.00	14.00	6.60	
80% protection	**	8.00	43.00	36.00	12.00	
Comparisons with other similar studies						**Reference**
Bermuda coastal fill	<10	0.12 - 18.4	3.8 - 204	0.02 - 0.07	0.05 -1.5	(Jones 2010)
Lyttelton Harbour / NZ	NA	<1.1 - 1.2	<4.2 - 8.8	NA	<1.1 - 2.7	(Sneddon 2011)
Lyttelton Harbour / NZ	44 - 699	0.30 - 1.63	0.48 - 6.10	0.009 - 0.048	0.06 - 0.84	Chapter 4 of this study
Thilafushi Island (EIA report)	0.11 - 0.32	NA	NA	0.07 - 0.14	0.20 - 0.44	(CDE Consulting 2011)
Background level	2	0.25	4.9	0.11	0.03	(Haynes 2014)

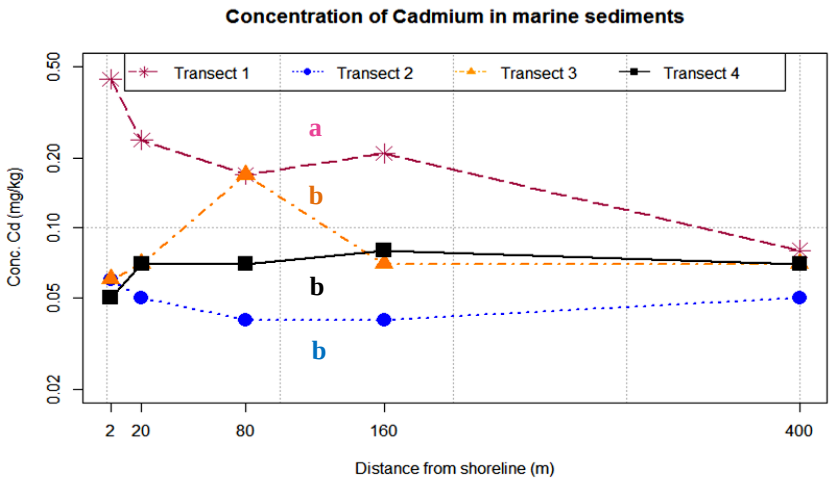
** Values not provided in the ANZECC guideline. MPI-metal pollution index. NA- trace elements not analysed. Data values in bold indicate concentrations exceeding the guideline value. Background levels of trace elements in seawater were obtained from CRC Handbook of Chemistry and Physics, Chapter 14 (Haynes 2014).

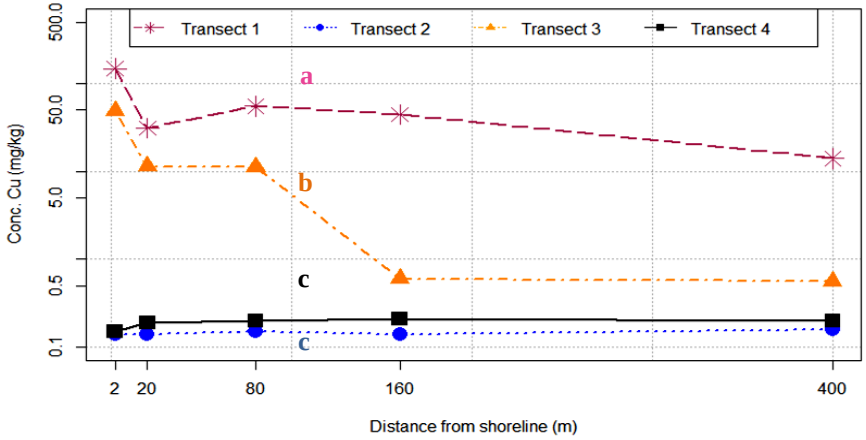
Table 3.2: Mean and range of trace elements concentrations in marine sediments, MPI values for each transect line, ANZECC guideline values and comparisons of trace element concentrations in the sediments with other similar studies

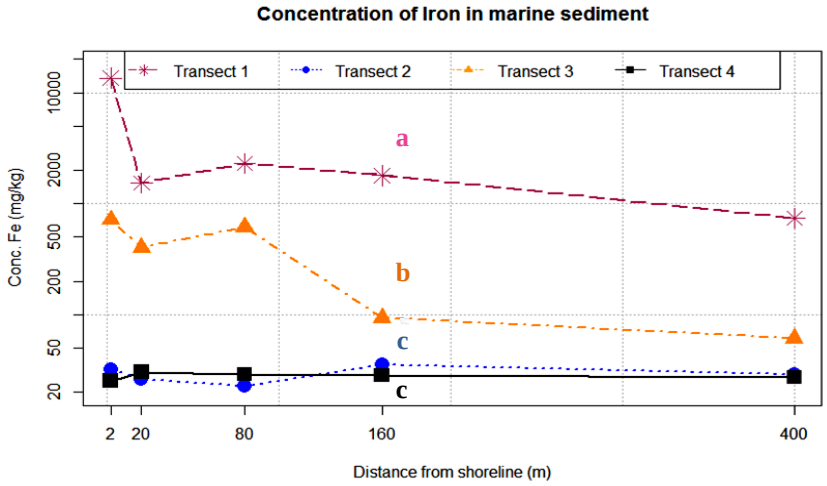
Sample	Fe	Cu	Zn	As (total)	Cd	Pb	MPI
Marine sediment (µg g dry wt^{-1})							
T1	3991 (741 - 13563)	58.70 (14 - **148**)	128 (20 - **426**)	4.70 (4 - 6)	0.23 (0.078 – 0.444)	19.52 (6 - 31)	29.29
T2	29.10 (23 - 35)	0.15 (0.14 - 0.16)	0.36 (0.23 - 0.47)	1.85 (1 - 3)	0.048 (0.038 - 0.057)	0.23 (0.20 – 0.26)	0.56
T3	377 (61 - 713)	14.56 (0.56 - 49)	6.25 (1 - 14)	2.19 (2 - 3)	0.088 (0.062 - 0.174)	2.01 (0.45 – 4.62)	4.87
T4 – reference site	28.03 (25 - 30)	0.19 (0.15 - 0.21)	0.26 (0.21 – 0.35)	1.82 (1 - 2)	0.068 (0.055 - 0.078)	0.39 (0.26 – 0.45)	0.64
ANZECC Interim Sediment Quality Guideline (ISQG) values for the protection of marine species (µg g dry wt^{-1})							
ISQG-low	**	65	200	20	2	50	
ISQG-high	**	270	410	70	10	210	
Comparisons with other similar studies							**Reference**
Thilafushi Island (EIA report)	<1.4 - 16	<0.4 – 5.46	<0.4 - 4.27	<0.2 – 8.04	0.08 – 0.98	<2 – 17.12	(CDE Consulting 2011)
Lyttelton sea-fill / NZ	NA	8 - 15	54 - 93	6 - 8	NA	22 - 49	(Sneddon 2011)
Kiribati	NA	0.3 - 14	1.2 - 77	< LOQ	< LOQ	3.4 - 13	(Redfern 2006)
Suva Harbour / Fiji	NA	59 - 306	88 - 670	0.7 - 45	59 - 306	19 - 272	(Naidu & Morrison 1994)
Bermuda	800- 11100	3 - 159	16.6 - 1380	2.7 - 29	3 - 159	15 - 259	(Jones 2010)
Tanapag Lagoon / Saipan	NA	0.22 - 28	1.63 - 127	1.33-10	<0.1 - 0.58	<0.4 - 41	(Denton et al. 2001)
Suva Harbour	46000 - 49000	99 -143	149 - 200	NA	NA	23	(Maata & Singh 2008)
Lyttelton Harbour / NZ	18199 - 32135	9 - 22	56 - 152	6 - 10	0.039 - 0.133	17 - 75	Chapter 4 of this study

** Values not provided in the ANZECC guideline. MPI-metal pollution index. NA- trace elements not analysed. LOQ- limit of quantification. Data values in **bold** indicate concentrations exceeding the guideline value.


Concentration of Arsenic in marine sediment
Transect 1
Transect 2
Transect 3
Transect 4
Conc. As (mg/kg)
1
2
3
4
5
6
7
a
b
b
b
2
20
80
160
400
Distance from shoreline (m)


Concentration of Cadmium in marine sediments
Transect 1
Transect 2
Transect 3
Transect 4
Conc. Cd (mg/kg)
0.02
0.05
0.10
0.20
0.50
a
b
b
b
2
20
80
160
400
Distance from shoreline (m)


Concentration of Copper in marine sediments
Transect 1
Transect 2
Transect 3
Transect 4
Conc. Cu (mg/kg)
0.1
0.5
5.0
50.0
500.0
a
b
c
c
2
20
80
160
400
Distance from shoreline (m)


Concentration of Iron in marine sediment
Transect 1
Transect 2
Transect 3
Transect 4
Conc. Fe (mg/kg)
20
50
200
500
2000
10000
a
b
c
c
2
20
80
160
400
Distance from shoreline (m)

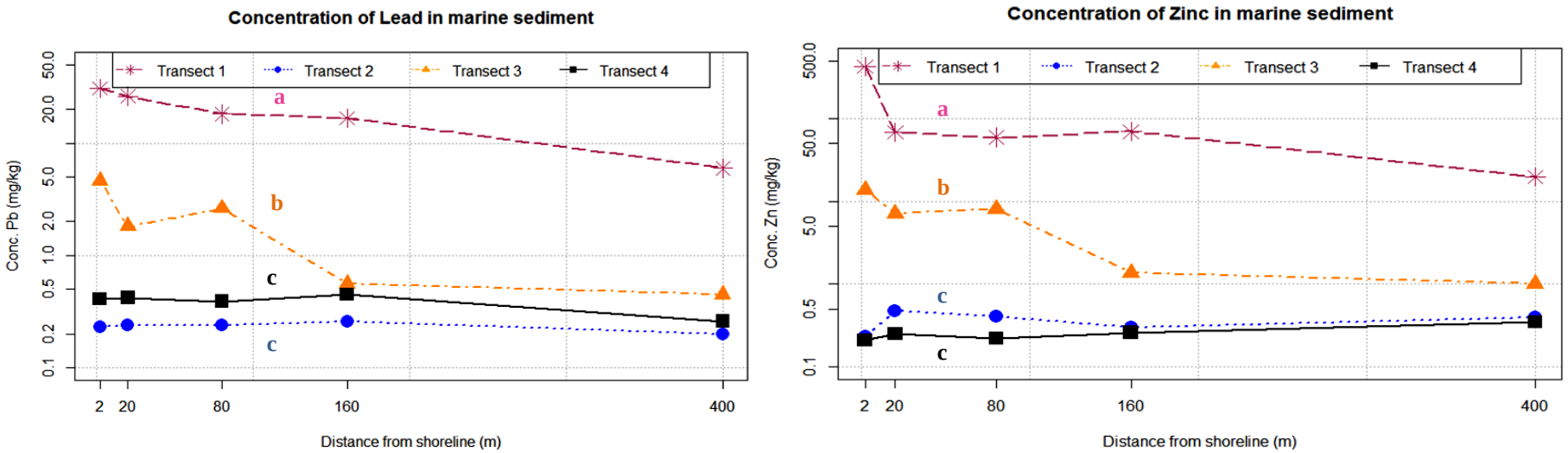


Figure 3.6: Trace element concentrations in marine sediments along the four transect lines. Transect lines sharing letters are not significantly different.

3.3.3 TRACE ELEMENT CONCENTRATIONS IN MARINE BIOTA

The trace element concentrations (mean values ± SE) in fish tissues are presented in Appendix A6 and the trace element concentrations (mean values ± SE) in shellfish, marine worms and green algae in Appendix A7. Comparison of trace element concentrations in different biota species and fish tissues from the two sites are presented in graphical form in Figure 3.7 and Figure 3.8, respectively. Trace element concentration data are presented on a wet weight basis. Statistical variation of trace elements between the biota species and sites are indicated in Figures 3.7 and 3.8.

3.3.3.1 FISH TISSUES

The results of combined fish tissues (muscle and organs) indicated that arsenic, cadmium, copper, iron, and zinc concentrations in red mullet were significantly higher than those of parrotfish. The overall tissue concentrations indicated that there were no significant differences in concentrations of copper and iron in red mullet or parrotfish between the two sites. Concentrations of zinc in red mullet from Thilafushi sea-fill site were significantly higher than those of the reference site, while parrotfish from the reference site contained significantly higher levels of zinc than those collected from Thilafushi sea-fill site. Red mullet of Huruelhi Island contained significantly higher concentrations of arsenic than those of Thilafushi Island, but there were no significant differences in arsenic levels between the two sites for parrotfish. There were no significant differences in cadmium concentrations between the two sites.

In general, trace element concentrations were significantly lower in muscle tissue than in liver, kidney and gonad. Overall trace element concentrations within the fish tissues were in the order: liver ≥ kidney ≥ gonad > muscle (Figure 3.8).

3.3.3.2 OTHER BIOTA

The result of the combined marine biota (muscle tissue was only included for fish) indicated that copper, iron, mercury, lead and zinc concentrations were significantly higher in Thilafushi Island (sea-fill) than Huruelhi Island (reference site). Conversely, arsenic and cadmium concentrations in biota were significantly higher for the reference site than for the sea-fill site.

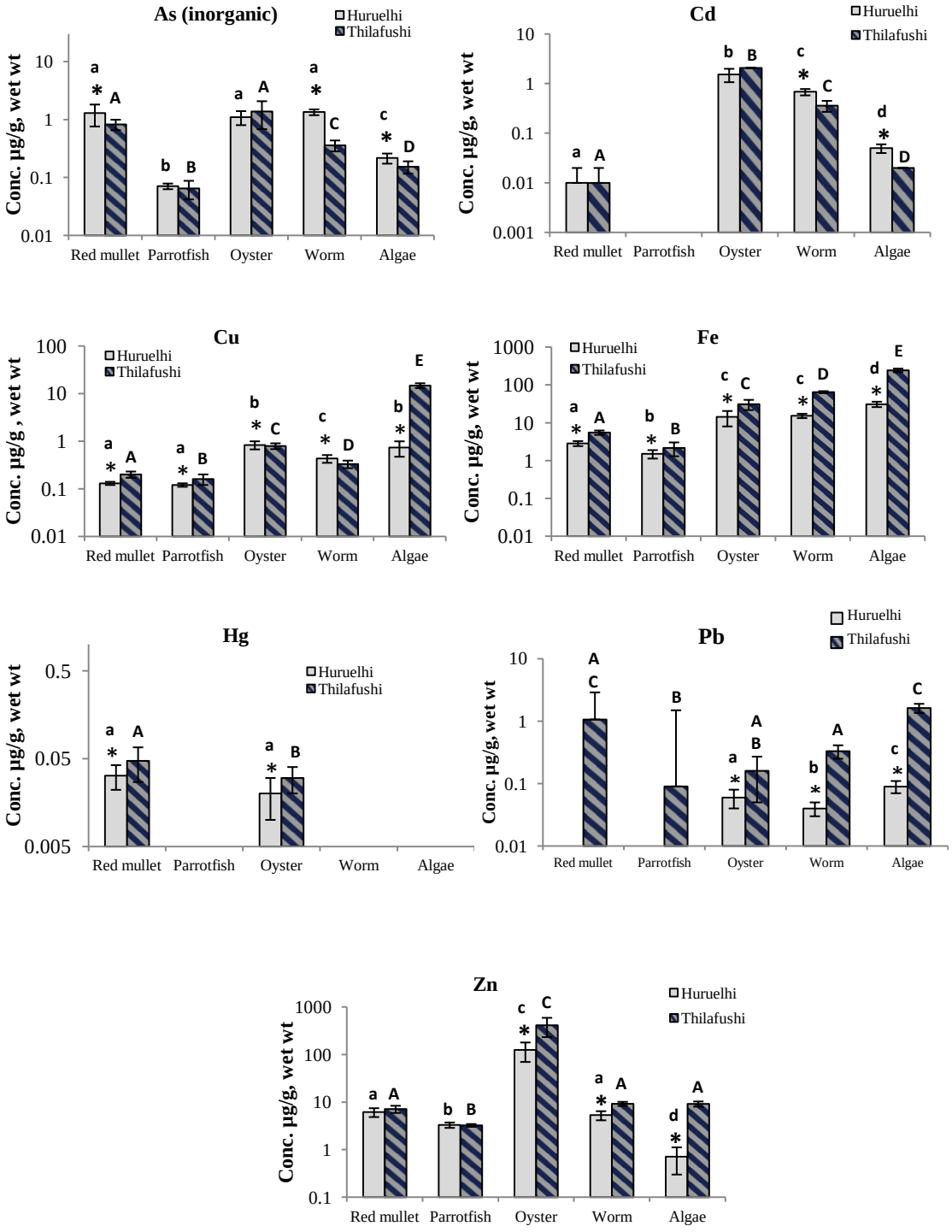
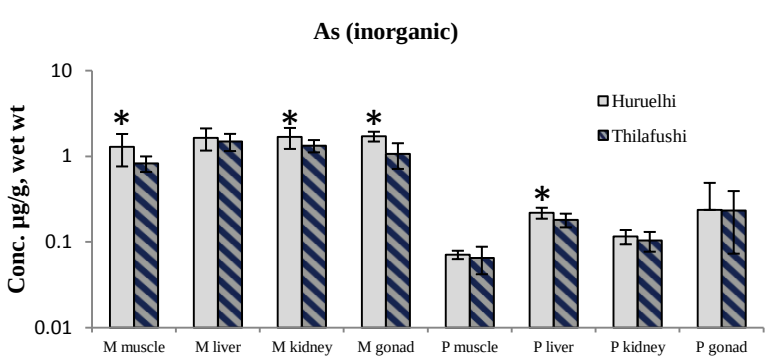
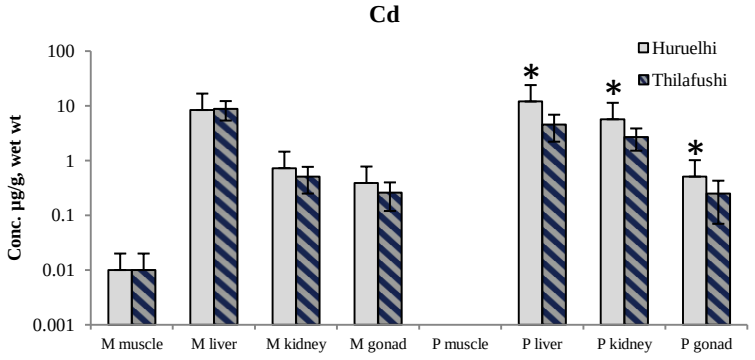
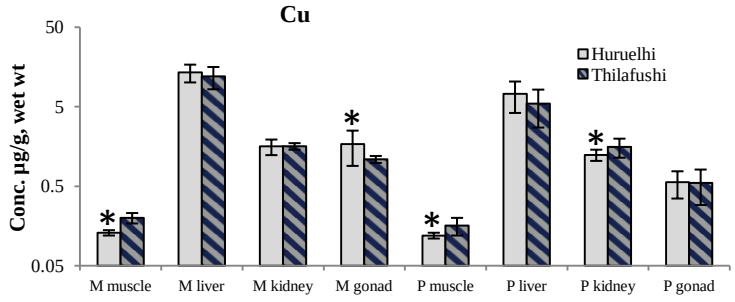
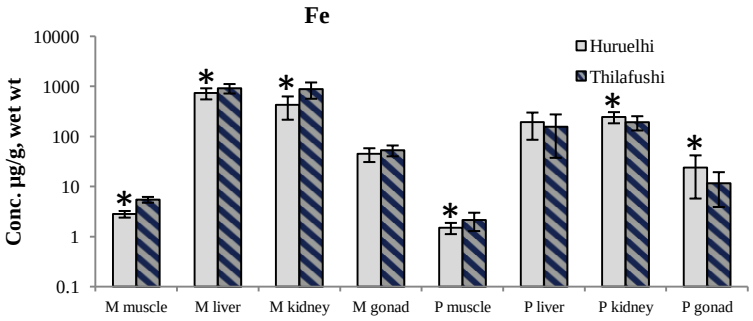


Figure 3.7: Comparisons of trace element concentrations in marine biota. Plotted values represent means ± standard errors (n = 12). Species sharing letters are not significantly different, and asterisks indicate significant differences between sites within a species. Lowercase letters represent Huruelhi samples and uppercase letters are for Thilafushi samples. Hg was < LOQ in parrotfish muscle, marine worms and algae of both sites, Pb was < LOQ in muscle of red mullet and parrotfish from Huruelhi Island, and Cd was < LOQ for parrotfish muscle of both sites.


As (inorganic)
Conc. µg/g, wet wt
10
1
0.1
0.01
Huruelhi
Thilafushi
M muscle
M liver
M kidney
M gonad
P muscle
P liver
P kidney
P gonad


Cd
Conc. µg/g, wet wt
100
10
1
0.1
0.01
0.001
Huruelhi
Thilafushi
M muscle
M liver
M kidney
M gonad
P muscle
P liver
P kidney
P gonad


Cu
Conc. µg/g, wet wt
50
5
0.5
0.05
Huruelhi
Thilafushi
M muscle
M liver
M kidney
M gonad
P muscle
P liver
P kidney
P gonad


Fe
Conc. µg/g, wet wt
10000
1000
100
10
1
0.1
Huruelhi
Thilafushi
M muscle
M liver
M kidney
M gonad
P muscle
P liver
P kidney
P gonad

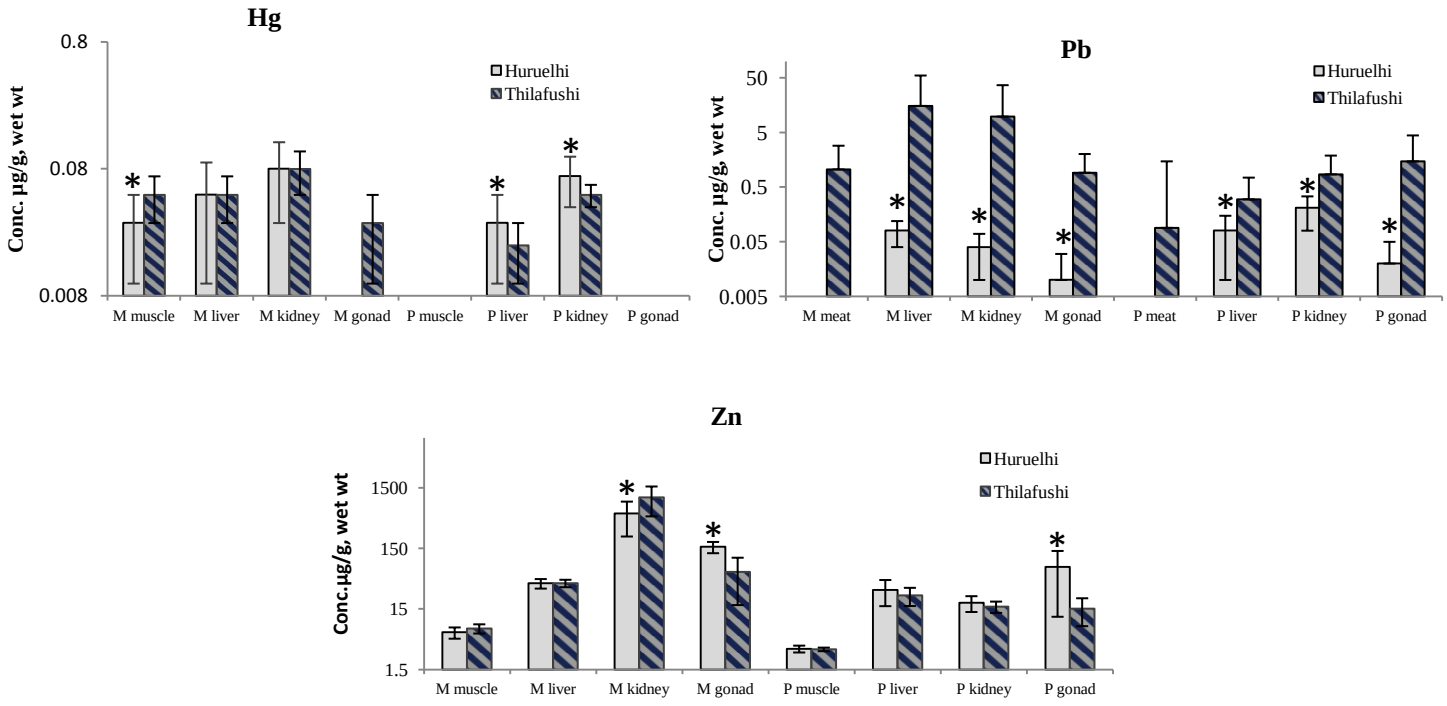


Figure 3.8: Comparison of mean trace element concentrations in fish tissues (P = parrotfish, M = red mullet). Plotted values represent means ± standard errors (n = 12). Asterisks indicate significant differences between sites within a tissue. Cd and Hg were <LOQ in parrotfish muscle from both the sites, while Pb was <LOQ in parrotfish muscle of Huruelhi Island. Hg was <LOQ in gonad of parrotfish from both the sites and mullet gonad from Huruelhi Island.

The highest mean concentrations of arsenic were measured in penguin wing oysters from Thilafushi, and the lowest in red mullet muscle tissue at the same site. The order of total arsenic concentrations between species was: red mullet = oyster > worm > algae > parrotfish for the sea-fill site; with red mullet = oyster = worm > algae > parrotfish for the reference site.

For cadmium, the highest mean concentrations were measured in penguin wing oysters from Thilafushi, and the lowest concentrations in muscle tissue of red mullet from the reference site. Cadmium was not detected in parrotfish muscle from either site. The overall order of cadmium concentrations between the species was: oyster > worm > algae > red mullet, for both Thilafushi and Huruelhi samples.

The highest mean concentration of mercury was measured in red mullet muscle from Thilafushi. Mercury was not detected in the marine worms, green algae or muscle tissues and gonad of parrotfish at either site. The order of mercury concentrations between the marine species was: mullet > oyster for Thilafushi samples, while mullet and oysters at the reference site had statistically indistinguishable levels of mercury.

Green algae from Thilafushi contained the highest mean concentration of lead, while the lowest mean concentration was measured in the peanut worms collected from the reference site. Lead was not detected in the muscle of red mullet or parrotfish of the reference site. The order of the lead concentrations in marine biota species were: algae ≥ mullet = worm = oyster > parrotfish for Thilafushi samples, and algae > oyster > worm for Huruelhi samples.

The highest mean concentration of copper was measured in green algae of Thilafushi, while the lowest was measured in parrotfish muscle of the reference site. The copper concentrations in collected species were in the order: algae = oyster > worm > mullet > parrotfish for Thilafushi samples, and algae = oyster > worm > mullet = parrotfish for Huruelhi samples.

Similar to the pattern exhibited for copper, the highest mean concentration of iron was obtained for green algae from Thilafushi, and the lowest mean concentrations in muscle of parrotfish from the reference site. The general order of iron concentrations was: algae > worm > oyster > mullet > parrotfish for Thilafushi

samples, and algae > worm = oyster > mullet > parrotfish for Huruelhi samples.

Penguin wing oysters from Thilafushi contained the highest mean concentration of zinc, while the lowest mean concentration was measured in green algae of the reference site. The concentrations of zinc followed the order: oyster > worm = algae = mullet > parrotfish for Thilafushi samples, and oyster > mullet = worm > parrotfish > algae for Huruelhi samples.

3.3.4 RISK ASSESSMENT FOR CONSUMPTION OF SEAFOOD

Trace element concentrations measured in the food chain species of this study were compared to the Food Standard Australia New Zealand (FSANZ) maximum allowable levels (MLs) as the principal referencing standard, and European Commission (EC) maximum allowable values and other NZ/Australia standards (from FAO data) (see Table 1.3) where FSANZ values were not available.

The concentrations of inorganic arsenic did not exceed the ML value for fish in any studied tissue (2 μg g wet wt^{-1}), and the concentrations in the penguin wing oysters also did not exceed the ML value for molluscs (2 μg g wet wt^{-1}). Although the cadmium concentrations in the muscle tissue of red mullet did not exceed the ML value for fish (0.05 μg g wet wt^{-1}; EC standard), liver, kidney and gonad concentrations did. The cadmium concentrations in liver of red mullet from both sites were over 150-fold higher than this ML value. Cadmium concentrations in the penguin wing oysters from the sea-fill site were at the ML value for molluscs (2 μg g wet wt^{-1}). Fish tissue mercury and copper concentration did not exceed the ML values (0.5 μg g wet wt^{-1}, and 30 μg g wet wt^{-1} for mercury and copper respectively) for either fish or molluscs. Lead concentrations in all tissues of red mullet from the sea-fill site, and gonad and kidney of parrotfish from the sea-fill site, exceeded the ML value for fish (0.5 μg g wet wt^{-1}). Zinc concentrations in fish muscle and liver tissues did not exceed the ML value of 40 μg g wet wt^{-1}. However, the gonad and kidney of red mullet from the sea-fill site, and gonad and kidney of parrotfish from the reference site, and shellfish from both the sampling sites did.

The estimated dietary exposures of the contaminants in the three risk assessment scenarios are presented in Tables 3.3 and 3.4. The values presented in

bold in Table 3.3 and Table 3.4 exceed the respective provisional tolerable weekly intake (PTWI) values. The italicised values represent the total weekly intake of the trace elements through consumption of fish muscle, fish organs and shellfish.

Table 3.3: Estimated weekly intake (EWI) (µg/kg body weight/week) of trace elements at different consumption rates by different age-gender cohorts at different contaminant concentrations (mean, 95th percentile) in fish muscle (Scenario one)

			EWI of trace elements from fish muscle (µg / kg BW / week)			
			As (inorganic)	Cd	Hg	Pb
WHO / JECFA PTWI values			**21**	**5.6**	**1.60**	**25**
Consumption rates g/week		Concentration levels				
65 kg (male)	560	Mean	7.1	0.07	0.40	9.16
		95th Percentile	9.4	0.15	0.73	**37**
	1750	Mean	**22**	0.22	1.3	**29**
		95th Percentile	**29**	0.48	**2.3**	**116**
	2940	Mean	**37**	0.37	**2.1**	**48**
		95th Percentile	**49**	0.81	**3.8**	**195**
49 kg (female)	560	Mean	9.5	0.09	0.53	12
		95th Percentile	12	0.20	0.97	**49**
	1750	Mean	**30**	0.29	**1.7**	**38**
		95th Percentile	**39**	0.64	**3.0**	**154**
	2940	Mean	**50**	0.49	**2.8**	**64**
		95th Percentile	**65**	1.07	**5.1**	**258**
30 kg (child)	560	Mean	15	0.15	0.87	20
		95th Percentile	20	0.33	**1.6**	**80**
	1750	Mean	**48**	0.47	**2.7**	**62**
		95th Percentile	**63**	1.04	**4.9**	**251**
	2940	Mean	**81**	0.79	**4.6**	**104**
		95th Percentile	**107**	1.74	**8.3**	**422**
13 kg (toddler)	560	Mean	**36**	0.35	**2.0**	**46**
		95th Percentile	**47**	0.77	**3.6**	**185**

Values in bold exceed the PTWI values

In the first scenario, cadmium consumption did not exceed the PTWI, regardless of the model parameters (Table 3.3). Similarly, using mean fish trace element concentrations, and the lowest fish consumption scenario, all values remained under the PTWI (for adults and children) for all elements for which EWI's were determined. The only exception to this was for the toddler, which exceeded PTWI values for inorganic arsenic, mercury and lead. Assuming higher fish consumption

rates, almost all groups exceeded the PTWI for arsenic, mercury and lead. Even at the lowest consumption rate, but using the 95th percentile trace element concentration value, the PTWI for lead in adult males and adult females, and lead and mercury for children was exceeded.

The second scenario (Table 3.4) resulted in increased EWI values for exposures, and toddlers exceeded the PTWI value of cadmium. In addition, all the BW groups exceeded PTWI value for inorganic arsenic and lead, while the female, child and toddler exceeded the PTWI value for mercury as well. Similarly, the EWI values were again increased for each BW group with the addition of shellfish in the third scenario (Table 3.4). With the addition of a very low rate of shellfish consumption, children exceeded the PTWI value for all elements.

Table 3.4: Estimated weekly intake (EWI) of trace elements by different age-gender cohorts for the consumption of fish muscle and organs (Scenario 2), and addition of shellfish (Scenario 3) at the mean concentrations of each tissue

			EWI of trace elements from fish muscle and organs (µg / kg BW / week)			
			As (inorganic)	Cd	Hg	Pb
	WHO / JECFA PTWI values		**21**	**5.6**	**1.60**	**25**
Scenario 2	Fish tissues	Consumption rate g /week				
Male	Muscle	1750	**22.27**	0.27	1.35	**28.54**
	Liver	8	0.18	1.09	0.01	1.90
	Gonad	11	0.18	0.04	0.01	0.16
	Total dietary intake from seafood		***22.63***	*1.40*	*1.36*	***30.59***
Female	Muscle	1750	**29.54**	0.36	**1.79**	**37.86**
	Liver	8	0.24	1.44	0.01	2.52
	Gonad	11	0.24	0.06	0.01	0.21
	Total dietary intake from seafood		***30.02***	*1.86*	***1.80***	***40.58***
Child	Muscle	1750	**48.24**	0.58	**2.92**	**61.83**
	Liver	8	0.40	2.36	0.01	4.11
	Gonad	11	0.39	0.10	0.01	0.34
	Total dietary intake from seafood		***49.03***	*3.04*	***2.94***	***66.28***
Toddler	Muscle	560	**35.62**	0.43	**2.15**	**45.66**
	Liver	8	0.92	5.44	0.03	9.49
	Gonad	11	0.90	0.22	0.03	0.78
	Total dietary intake from seafood		***37.45***	***6.09***	***2.21***	***55.93***
Scenario 3						
	Fish tissues and shellfish	Consumption rate g/week				
Male	Muscle	1750	**22.27**	0.27	1.35	**28.54**
	Liver	8	0.18	1.09	0.01	1.90
	Gonad	11	0.18	0.04	0.01	0.16
	Shellfish	43	0.91	1.37	0.02	0.11
	Total dietary intake from seafood		***23.54***	*2.77*	*1.38*	***30.70***
Female	Muscle	1750	**29.54**	0.36	**1.79**	**37.86**
	Liver	8	0.24	1.44	0.01	2.52
	Gonad	11	0.24	0.06	0.01	0.21
	Shellfish	43	1.21	1.82	0.03	0.14
	Total dietary intake from seafood		***31.23***	*3.68*	***1.83***	***40.72***
Child	Muscle	1750	**48.24**	0.58	**2.92**	**61.83**
	Liver	8	0.40	2.36	0.01	4.11
	Gonad	11	0.39	0.10	0.01	0.34
	Shellfish	43	1.98	2.97	0.04	0.23
	Total dietary intake from seafood		***51.01***	***6.00***	***2.98***	***66.51***

Values in bold exceed the PTWI values, and the values in italics are the total amount of weekly intake of trace elements via consumption of fish muscle, organs and shellfish.

3.4 DISCUSSION

3.4.1 ENVIRONMENTAL CHARACTERISATON

The pH range of seawater from Thilafushi and Huruelhi Islands was in agreement with typical coastal water pH (Byrne 2002; Sneddon 2011), and with previously measured pH values for Thilafushi coastal water by CDE Consulting (2011). The pH is an important factor determining remobilisation of trace elements in ecosystems. A lower pH (i.e. more acidic) seawater often indicates higher concentrations in the overlying water column (Denton et al. 1997; Williamson et al. 2003), and thus higher bioavailability of trace elements (Byrne 2002). In future, measurements of sediment porewater would be valuable to determine if pH was exerting an influence on sediment-trace element interactions, and whether this could therefore be a significant contributor to environmental trace element distribution.

At transect lines T1, T2 and T3 the concentrations of iron were the highest among all the trace elements in seawater followed by zinc, copper, lead and cadmium. This order is in agreement with contaminant monitoring studies at the Bermuda marine fill and the sea-fill site of Lyttelton Harbour of New Zealand (Jones 2010; Sneddon 2011). However, the order of trace element concentrations in seawater at the T3 (boat yard) site were slightly different, with copper higher than zinc. This could be due to the use of copper as an antifouling paint (Denton et al. 1997). The order of the trace elements concentration in background seawater is considered to follow the order: zinc > iron > copper > cadmium > lead (Haynes 2014), a trend that is different from what was measured for the sea-fill site and the reference site. Differences are likely due to the source geology at any given sampling site (Haynes 2014).

A limited number of studies on trace element concentrations in seawater of coastal landfill or sea-fill sites have been performed (Table 3.2). A previous study carried out at Thilafushi Island (CDE Consulting 2011) reported concentrations of cadmium and lead in seawater comparable to those measured here, while the iron concentrations were lower than those of the current study. Lead and zinc concentrations at the Bermuda marine fill (Jones 2010) were higher than those of this study, while iron, copper and cadmium were comparable. Copper concentrations in the seawater of Thilafushi Island in this study were comparatively higher than those

of Lyttelton Harbour sea-fill site. These differences in trace element concentrations in seawater at different sea-fill sites could be due to a number of factors. For example, variations in the nature of the fill materials, trace element leaching rates, differences in the geology, the presence of other anthropogenic sources of trace elements in the surrounding environment (e.g. boating or industrial activities at a close proximity), sewage discharges and storm water runoff could all influence environmental trace element concentrations (Denton et al. 1997; Jones 2010; Maata & Singh 2008; Morrison & Brown 2003; Naidu & Morrison 1994).

Trace element concentrations in seawater and marine sediments were compared with ANZECC guideline values (Table 3.1 and 3.2), as guidelines specific to the Maldives are not available. In general, seawater samples from all three transect lines at Thilafushi Island sea-fill exceeded the ANZECC trigger value for the protection of 99% of marine species against copper toxicity. The copper concentrations in seawater at the transect line T1 exceeded ANZECC trigger values for marine species at the 95% protection level, while the concentrations at T3 exceeded the 80% protection level (Table 3.1), implying that these levels of copper can pose risks to at least 20% of the aquatic species in these sites. Similarly, copper concentrations in the marine sediments of T3 exceeded the ANZECC guideline value of ISQG-low; while sediment zinc concentrations exceeded the ISQG-high value at transect line T1. This again implies that copper concentrations were high enough to trigger possible biological effects, with sediment zinc also likely to cause toxicity to sediment-dwelling biota at the sea-fill site.

The overall concentration of trace elements in the sediments followed the order: iron > zinc > copper > lead > arsenic > cadmium for the sea-fill site, and iron > arsenic > lead > zinc > copper > cadmium for the reference site. For the contaminated sea-fill site (specifically, transects T1 and T3) this pattern is consistent with monitoring carried out at the Lyttelton Harbour sea-fill site (Sneddon 2011), the study of marine landfill beside a coral reef in Bermuda (Jones 2010), and other similar studies (Glasby et al. 1988; Williamson 1992).

The order of trace element concentrations at the reference site (T4) of this study and the transect line T2 of Thilafushi Island (the sand-reclaimed site) was different from T1 and T3 (Table 3.2). The higher concentrations of arsenic than zinc

and copper at T2 and T4 suggest that this could be the typical trend for trace elements in the natural geological environment of Maldives. In general, the concentrations of trace elements in transect line T2 and transect line T4 were similar, most likely due to the clean sand reclamation area adjacent to the transect line T2, reflecting a lower level of contamination, akin to the reference site (T4).

One obvious reason for the significantly higher concentrations of trace elements in seawater and sediments of T1 and T3 compared to T2 at Thilafushi could be the higher input of trace elements from MSW fill activities. However, these sampling sites (T1 and T3) were near other point sources, including the boatyard at T3, discharges from commercial vessels, and a variety of scrap metals and racked/broken boats in the inner lagoon of the T1 transect. Numerous studies have shown that increased input of trace elements results in an increase in local sediment concentrations (Denton & Morrison 2009; Denton et al. 2001; Glasby et al. 1988; Jones 2010; Luoma 1990; Naidu & Morrison 1994; Redfern 2006; Williamson et al. 2003; Williamson 1992).

Another reason for the higher trace element concentrations in the inner lagoon could be due to the semi-enclosed and stagnant nature of this water body. The lower hydrodynamic energy facilitates deposition of finer particles and restricts movement of input materials, leading to local enhancement of trace elements (O'Connor & Ehler 1991; Williamson & Wilcock 1994). In addition, the higher concentrations of trace elements at T1 and T3 could be due to the comparatively finer nature of the sediment particles observed for all the sediments of the inner lagoon, and some of the T3 sediments. Fine particulates have a tendency to bind relatively more trace elements than more coarse particulates (Williamson et al. 2003). Arguing against this, however, sediments from the inner lagoon contained a higher proportion of larger particles compared to the other sites (CDE Consulting, 2011). However, the available organic carbon data for Thilafushi sediments (CDE Consulting 2011) showed that the sediments of inner lagoon contained a higher percent of organic carbon (0.29%) compared to the other sites (0.17-0.23%), which could account for the higher trace element content at the inner lagoon, as sediments with higher organic carbon are known to provide more binding sites for trace elements (Williamson et al. 2003).

Comparison of the current study with other sea-fill data (e.g. Suva Harbour,

Fiji (Maata & Singh 2008) and Bermuda (Jones 2010)), showed that the levels of some trace elements were comparable (e.g. copper), but other elements were not (Table 3.2). This could be because of the different nature of the fill materials, the rate of input or proximity to the point source, the geology of the site, and/or differences in physical transport characteristics (sea current and sediment movements). In addition, the chemical and physical properties of the sediment such as the organic content, the grain size or the sediment texture are important factors for determining the trace element concentrations in sediments (Jones 2010; Luoma 1990; Redfern 2006; Williamson 1992). Furthermore, the potential for redox reactions, the pH and the mineralogy of the site (e.g. the iron and manganese oxide content which influences binding and remobilisation), all play important roles in shaping environmental trace element patterns (Jones 2010; Luoma 1990; Redfern 2006; Williamson 1992). In general, the concentrations of trace elements in the Maldives sediment were more similar to those of Kiribati and Saipan than the other studies mentioned in Table 3.2. This similarity could be due to the related nature of the sediment, which mainly consists of coral-derived materials (Denton et al. 2001; Redfern 2006).

The iron concentrations measured in the sediments of the inner lagoon were several-fold higher than those of other sites in this study. In addition, the mean iron concentrations measured for the inner lagoon in this study were over 200-fold higher than the highest concentrations of iron measured by CDE Consulting (2011) for Thilafushi lagoon. The likely reason for the high concentrations of iron measured in the inner lagoon samples of this study are rusted and broken iron bars that have been directly exposed to seawater. Rusted metal bars and debris were observed lying around the boatyard near the shoreline of transect line T3.

The concentrations of iron, copper, zinc and arsenic in the sediments of this study were comparatively higher than those measured previously for Thilafushi Island (CDE Consulting 2011), while that same study reported comparatively higher concentrations of cadmium. The concentration of cadmium presented in the EIA report (CDE Consulting 2011) for the inner lagoon (0.98 mg kg^{-1}) was over two-fold higher than the highest value of cadmium measured in this study for the same location (0.44 mg kg^{-1}). Although the inner lagoon was a site common to both studies, the likely reason for the vast difference of some trace element concentrations could be

differences in distance of the sampling locations to the input sources.

Because there are multiple sources of trace elements at Thilafushi Island, and owing to the limited number of samples collected for this study, it is difficult to draw a definite conclusion that the observed elevated trace elements were a consequence of MSW. However, numerous studies on trace elements in marine sediments of coastal landfills and sea-fills indicate that fill activities significantly contribute trace elements to the surrounding marine environment (Denton & Morrison 2009; Jones 2010; Maata & Singh 2008; Naidu & Morrison 1994).

3.4.2 TRACE ELEMENTS IN MARINE BIOTA

Higher concentrations of copper, iron, mercury, lead and zinc were measured in the biota from the sea-fill site compared to the reference site. This is likely due to the higher environmental levels of these elements at the sea-fill location. The MPI values obtained from seawater and sediment concentrations indicated that the trace element loads were several fold higher at transect lines T1 and T3 compared to T4 at the reference site. Surprisingly, the arsenic and cadmium concentrations were higher at the reference site, even though there are no known anthropogenic sources of trace elements at this location. Huruelhi Island is a dumping-free zone, with minimal tourist activity. As suggested above this phenomenon could be a consequence of differences in natural base-line levels of trace elements between the two sites.

In general, the levels of all trace elements measured in fish tissues displayed the following order: liver ≥ kidney ≥ gonad > muscle. It is normal to find several-fold higher concentrations of trace elements in liver, kidney and gonad relative to fish muscle (Andres et al. 2000; Brooks & Rumsey 1974; Yılmaz et al. 2010). Trace elements preferentially accumulate at higher levels in these tissues as they are metabolically active (Allen 1995; Canli & Atli 2003; Romeo et al. 1999; Ünlü et al. 1996; Yilmaz 2003). Liver is considered the main storage and detoxification organ, while kidney is considered an excretory organ, and is thus a site where trace elements may accumulate prior to elimination from the body. At a biochemical level, the variation in trace element concentrations in different tissues of fish could be explained by the presence of metallothioneins. These are metal binding proteins with roles that include detoxifying trace elements (Rainbow 2002; Rainbow et al. 2006a; Roesijadi

1981; Wang & Rainbow 2010). The binding of metallic trace elements to metallothionein renders them detoxified (Pourang et al. 2004; Roesijadi 1992; Wang & Rainbow 2010), allowing them to accumulate. This phenomenon is then reflected in the increased tissue burdens in metabolically active tissues such as the liver, kidney and gonad, where metallothionein induction is especially prominent (Amiard et al. 2006; Engel & Brouwer 1984; Marie et al. 2006; Rainbow 2002; Wang & Rainbow 2010).

Parrotfish generally displayed lower trace element concentrations than all other species investigated in this study. Previous studies have shown that the concentrations of trace elements in different fish species are a result of different ecological needs, the contamination levels in the surrounding environment, feeding patterns, swimming behaviours and rate of metabolism (Canli & Atli 2003; Romeo et al. 1999; Young et al. 1980).

Although trace element concentrations measured in muscle of the two fish species in this study were generally comparable to those previously reported for comparable fish species (Table 3.5), arsenic (samples from both sites) and lead (samples from sea-fill site only) concentrations in red mullet of this study were higher than those reported in the literature for similar species. The higher concentration of arsenic could be related to the differences in species-specific physiological adaptations in handling trace elements. This concept is supported by the fact that red mullet from the reference site of this study also contained high concentrations of arsenic. Similar species-specific differences in arsenic handling were shown by Amlund and colleagues (2006). These authors showed that there were significant differences in tissue distribution and excretory patterns between fish species administered with arsenobetaine (Amlund et al. 2006).

The high concentration of lead measured in the red mullet of this study (sea-fill site) could be related to the higher environmental exposure of lead at the sea-fill site (see Section 3.3.2.2). Again, this hypothesis is supported by the trace element tissue burdens of red mullet at the reference site, which were significantly lower in lead than those of the sea-fill site. This is also reinforced by a previous study that investigated two fish species from contaminated sites in the Mediterranean Sea, which indicated that mullet (*Mugil cephalus*) from more contaminated sites contained

significantly higher concentrations of lead than those from less contaminated sites (Yilmaz 2003). The same study also reported that mullet accumulate more lead than another species of fish (*Trachurus mediterraneus*) from the same site (Yilmaz 2003). The higher concentrations of lead in red mullet from the sea-fill site of this study could also be related to its diet, as comparatively higher concentrations of lead were measured in the green algae and the marine worms from the sea-fill site. These items are an important part of mullet diet (Vassilopoulou & Papaconstantinou 1993).

Table 3.5: Comparisons of trace element concentrations (concentration range common to both sites for each species) in fish in µg g wet wt $^{-1}$ with other studies

Species	Total As	Cd	Cu	Fe	Hg	Pb	Zn	Ref
Red mullet (*Parupeneus indicus*)	5.65 - 11.48	<LOQ - 0.020	0.15 - 0.25	4.39 - 6.98	<LOQ - 0.09	0.05 - 6.35	5.61 - 9.19	This study
Parrotfish (*Scarus ventula)*	0.35 - 1.02	<LOQ	0.12 - 0.26	1.16 - 3.78	<LOQ	<LOQ - 0.47	2.87 - 3.52	This study
Wrasse (*Notolabrus fucicola*)	0.52 - 5.14	<LOQ	0.10 - 0.16	1.47 - 4.27	0.16 - 0.38	<LOQ	3.28 - 5.27	Chapter 6
Spotty (*Notolabrus celidotus*)	1.23 - 5.30	<LOQ	0.10 - 0.19	1.03 - 2.52	0.03 - 0.15	<LOQ	3.69 - 5.94	Chapter 6
Gurnard (*Trigla kumu*)		0.008 - 0.024	0.15 - 0.75	2.1 - 13.0		0.13 - 0.40	2.5- 16.2	1
Snapper (*Chrysophys auratus*)		0.002 - 0.015	0.03 - 0.50	1.0 - 12.0		0.15 - 0.60	2.0 - 10.0	1
Yellow fin bream (*Acanthopagrus australis*)	0.1 - 2.4	0.03 - 0.07	0.1 - 2.0		0.03-0.81	0.3 - 1.7	1.6 - 13.0	2
Sea mullet (*Mugil cephalus*)	0.1 - 3.8	0.02 - 0.08	0.2 - 2.8		<0.14	0.2 - 4.1	0.5 - 13.9	2
Snapper (*Chrysophrys auratus*)	0.4 - 4.4	0.01 - 0.09	0.2 - 1.5		0.06 - 1.94	0.2 - 1.5	5.30	2
Snapper (*Pagrus auratus*)	2.5 - 12.1	0.02	0.2 - 0.3		0.09 - 0.20	0.05	3.1-7.5	3
Blue cod (*Parapercis colias*)					0.07			4
Red cod (*Pseudophycis kachus*)					0.09			4
Sardine (*Sardina pilchardus*)	0.17- 0.96	0.003			0.009 - 0.067	0.004 -0.034		5
Red mullet (*Mullus surmuletus*)	0.23 - 0.73	0.002			0.041 - 0.139	0.230 -0.729		5
Sea bream (*Pegellus erythrinus*)		0.01 - 0.03			0.05 - 0.70	0.05 - 0.09		6
Stipped mullet (*Mullus barbatus*)		0.01 - 0.04			0.05 - 2.76	0.04 - 0.18		6
Malabar anchovy (*Thryssa malabarica*)		0.7	4.4		0.01	<1		7
Trevally (*Caranx georgianus*)		<LOQ - 0.62	<LOQ -0.7	5 - 11	0.02 - 0.08	<1	2.0 - 5.0	7
Bluespot mullet (*Valamugil seheli*)		0.17	6.35		<0.05	0.28	50.3	8
Blue tail mullet (*Valamugil buchanani*)		0.36	11.1		0.07	0.09	83.7	8
Red snapper (*Lutjanus vita*)		0.23	26.6		0.35	0.07	234	8
Sea mullet (*Mugil cephalus*)			0.69	58.25		10.87	42.18	9
Horse mackerel (*Trachurus mediterraneus*)			0.66	38.89		1.01	19.23	9
Green chromide (*Etroplus suratensis*)	<LOQ	1.32	2.19		<LOQ	0.23	12.3	10
Rohu (*Labeo rohita*)	<LOQ	0.02	14.7		<LOQ	0.32	12.3	10

LOQ-Limit of quantification; Ref: (1)- (Brooks & Rumsey 1974), (2)- (Bebbington et al. 1977), (3)- (Fabris et al. 2006), (4)- (Love et al. 2003), (5)- (Olmedo et al. 2013), (6)- (Storelli 2008), (7)- (Kureishy et al. 1981; Kureishy et al. 1983) (8)- (Agusa et al. 2007), (9)- (Yilmaz 2003), (10)- (Sivaperumal et al. 2007).

With respect to mercury concentrations in the current study, only red mullet and penguin wing oysters (at both sampling sites) displayed values above the LOQ. The higher concentration of mercury in red mullet could be due to this fish feeding at a slightly higher trophic level than the other organisms in this study. Concentrations of total mercury in red mullet were comparable to most species of fish in Table 3.5, suggesting similar trophic positions, age, size or similar physiological strategies for handling trace elements, such as rate of assimilation, detoxification, and excretion (Rainbow 2002; Reinfelder et al. 1998; Wang 2002; Wang & Rainbow 2008). Although the penguin wing oysters are filter feeders (lower trophic level), the measurable concentrations of mercury in these bivalves could be related to their large size (see below).

The concentrations of copper, iron and zinc measured in biota of this study were comparable to those previously reported (Table 3.5 and 3.6). The overall tissue concentration of copper and iron in the fish species from the two sites were not significantly different. This could be because these elements are essential to organisms and subject to regulation within a certain optimal range (Reinfelder et al. 1998; Yılmaz et al. 2010). However, zinc concentrations in red mullet from the sea-fill site were significantly higher than those of the reference site; while zinc concentrations in parrotfish of the reference site were significantly higher than those of the sea-fill site. In addition to the higher concentrations of zinc at the sea-fill site, another possible reason for the higher concentrations of this essential element in the red mullet is the reproductive cycle of these fish. Zinc is known to play an important role in the reproductive cycle and levels of this element increase during spawning (Banks et al. 1999; Miramand et al. 1991; Olsson et al. 1987).

Factors known to contribute towards differences in the accumulated trace element levels in different species of marine organisms include the trace element concentrations in the available diet (as observed in this study), the physiology of the species, the size and age of species, the position in the food chain, and the nature of the trace element (Fabris et al. 2006; Falconer et al. 1983; Reinfelder et al. 1998; Wang & Rainbow 2008). For instance mercury is an element known to present at higher concentrations in organisms at higher trophic positions, and in general mercury concentrations also increase with age and size (Bowles et al. 2001; Fabris et al. 2006;

Monteiro & Lopes 1990; Storelli & Marcotrigiano 2000; Storelli et al. 2002; Wang 2002; Zhu et al. 2012).

Animals are well adapted to regulate trace element burdens. Strategies include altering rates of ingestion, assimilation and excretion (Reinfelder et al. 1998; Wang & Rainbow 2008). For example, one important mechanism for the regulation of essential trace elements is by adjusting the efflux rate, to match the influx rate, thus allowing the animal to maintain concentrations at the desired optimal range (Reinfelder et al. 1998). Furthermore, some marine organisms store assimilated trace elements as insoluble metal rich granules and then eliminate them through the alimentary tract as faeces, while other organisms store the excess trace elements in kidneys and excrete them through urinary processes (Reinfelder et al. 1998; Wang & Fisher 1998). Which strategy is used will influence tissue burdens and overall rates of accumulation.

In summary, the concentration of trace elements in marine food chains depends on several factors including the physicochemical properties of the habitat (e.g. water chemistry, pH, metal speciation, availability), feeding ecology, and strategies for metal handling and storage adopted by the species therein (Blackmore & Wang 2004; Otero-Romani et al. 2005; Rainbow 2002,2007). Bioaccumulation, trace element handling strategies by different marine species, and trophic transfer of trace elements in coastal food chains are discussed in further detail in Chapter 5.

Table 3.6: Comparisons of trace element concentrations (concentration range common to both sites for each species) in penguin wing oysters, marine worms and green algae in µg g wet wt $^{-1}$ (µg g dry wt $^{-1}$) with other studies

Species	Total As	Cd	Cu	Fe	Hg	Pb	Zn	Ref
Bivalve shellfish								
Penguin Wing Oyster (*Pteria penguin*)	5.1 -24 (27 -127)	1.1 - 4.5 (6.9 - 24)	0.85 - 1.1 (4.4 - 6.1)	19 - 53 (103 - 283)	0.02 - 0.04 (0.12 - 0.24)	0.07 - 0.45 (0.36 - 2.4)	52 - 532 (278 - 2105)	This study
Green-lipped mussel (*Perna canaliculus*)	(8.7 - 12.0)	(0.26 - 0.73)	(3.3 - 4.8)	(310 - 972)	(0.10 - 0.22)	(0.44 - 1.9)	(46.1 - 100)	Chapter 5
Green-lipped mussel (*Perna canaliculus*)		0.10 - 1.00	0.2 - 28.0	26 - 280	0.04 - 0.19	0.1-7.8	0.5 - 28.0	1
Black clam (*Villorita cyprinoides*)	<LOQ	0.05	3.9		<LOQ	0.32	19	2
Abalone (*Haliotis rubra*)	6.4 - 13	0.10 - 0.18	0.6 - 6.3		0.01 - 0.02	0.05 - 0.06	8.3 - 13	3
Oysters (*Crassostrea virginica*)	(10)	(4.11)	(146)	(294)	(0.13)	(0.64)	(2150)	4
Oyster (*Saccostrea glomerata*)		(1.6 - 15)	(219 - 1413)	(97 - 1146)			(998 - 8629)	5
Marine worm								
Sipunculid worm (*Sipunculus indicus)*	2.6-16 (13 - 87)	0.22 -0.56 (1.2 - 1.3)	0.28 - 0.44 (1.1 -1.3)	44 - 51 (198 -211)		0.20 - 0.44 (0.91 -2.0)	7.8 -11 (38 - 42)	This study
Polychaete worm	(21.6-77.3)	(1.6- 3.6)	(3.4 - 5.9)	(221 - 465)		(0.17 - 0.59)	(120 - 269)	Chapter 5
Polychaete worm	(2.0 -14.8)							7
Polychaete worm	(8.8-117)	(<0.2 - 0.6)	(9.4 - 858)	(427-1521)		(2.1-34.5)	(69 - 201)	8
Green algae								
Chlorophyceae	0.97 - 2.5 (5.3 - 11.1)	0.02 – 0.7 (0.09 -0.32)	12.7 -17.4 (64 -75)	204 -290 (1006 -1185)		1.08 -1.80 (5.6 -7.2 0	7.1 – 10.7 (37 - 47)	This study
Chlorophyceae	(8.3 - 17.4)	(0.05 - 0.06)	(9.7 - 10.8)	(9007 - 3919)		(6.3 - 29.8)	(14.5 - 36.0)	Chapter 5
Chlorophyceae		(0.1 - 2.5)	(1.1 - 4.3)	(84.7 - 119.3)		(2.1 - 5.5)	(39.0 - 82.5)	9
Chlorophyceae	0.4 - 3.9							10

LOQ-Limit of quantification; Ref (1)- (Nielsen & Nathan 1975), (2)- (Sivaperumal et al. 2007), (3)- (Fabris et al. 2006) (4)- (Presley et al. 1990), (5)- (Phillips 1979), (7)- (Watts et al. 2013), (8)- (Rainbow et al. 2006a), (9)- (Haritonidis & Malea 1999), (10)- (Sanders 1979)

3.4.3 RISK ASSESMENT FOR CONSUMPTION OF SEAFOOD

Although the concentrations of inorganic arsenic, mercury, copper and zinc in the muscle tissues of fish in this study did not exceed limits considered to be protective of human health, cadmium concentrations measured in fish liver and gonad, and in shellfish were high enough to suggest health risks from regular consumption. The concentrations of zinc in the gonads were also high enough to potentially cause harm if consumed regularly. Furthermore, concentrations of lead in fish muscle, liver and gonad also exceeded ML values. Overall, these findings suggest that consumption of red mullet from the sea-fill site, and consumption of any fish organ from this site, is potentially hazardous for human health.

The weekly intake of inorganic arsenic, mercury and lead can exceed the PTWI values in different consumption scenarios in all BW groups. In addition, children and toddlers can exceed the PTWI values for cadmium under different exposure scenarios in this study. The calculated EWI of trace elements in this study was several times higher than those reported for Spain, Italy, New Zealand, Cambodia, Malaysia, Thailand and Indonesia (Agusa et al. 2007; NZTDS 2009; Olmedo et al. 2013; Storelli 2008) for fish with similar trace element concentrations. This is because Maldivians consume a high quantity of fish relative to other parts of the world. Moreover, fish organs including liver and gonad are considered to be delicacies in the Maldives, exacerbating potential risk. The diets of Maldivians are mainly based on starchy foods items including a large proportion of rice and flour (Golder et al. 2001). Rice can also be a source of cadmium and arsenic, which may further result in intake rates that exceed the PTWI values for these elements in the Maldives (McLaughlin et al. 1999; Wang et al. 2013).

Since there were limited numbers of species investigated in this current study, and no trace element data are currently available for any food items in the Maldives, it is extremely important that further studies of trace element concentrations in seafood species are carried out in the Maldives, with special attention being given to items sourced from Thilafushi Island. Although red mullet and parrotfish are comonly consumed species in the Maldives, there are other species that are more widely consumed. These include snapper (Lutjanidae), grouper (Serranidae), jack (Carangidae), bigeye scad (*Selar crumenophthalamus*), Indian mackerel (*Rastrelliger*

kanagurta), bait fish species and deep water fish including tuna and swordfish (Adam 1995; Kawarazuka & Béné 2011). Previous studies have reported that fish from the same study site can contain significantly different concentrations of trace elements (Bebbington et al. 1977; Brooks & Rumsey 1974; LeBlanc & Jackson 1973; Love et al. 2003; Yilmaz 2003), a finding also shown in the current study when comparing parrotfish and red mullet. Therefore, the results obtained from this study could under- or over-estimate true exposure. Nevertheless the risks to the community due to trace element intake via fish consumption are likely to be significant. Of particular concern is the high level of lead found in the red mullet from the sea-fill site. Lead toxicity can cause wide range of impacts on human such as impairment of red blood cell synthesis, kidney disorders, hypertension, hyperactivity, bone defects, learning disabilities, disruption to metabolic pathways, neurological disorders and brain damage (GESAMP 1985; O'Neill 1998).

3.5 CONCLUSIONS

Some of the trace element concentrations in seawater, marine sediments and biota of Thilafushi Island exceeded regulatory limits for the protection of marine species and/or human consumers of these organisms. The risks associated with consumption of seafood from the Thilafushi Island sea-fill site are likely to be high, even at normal consumption rates. This is especially true for lead and cadmium, which exceeded the maximum allowable levels set by regulatory authorities for seafood. In general, the results suggest that the disposal of MSW at the sea-fill can increase the dietary exposure of mercury and lead, and exposures exceed the JECFA tolerable weekly intakes for these elements. The results also show that even if the concentrations in seafood meet the existing maximum allowable levels, people can still exceed the PTWI values due to the high rate of fish consumption in the Maldives. Therefore, it is advisable for regulatory authorities to establish lower maximum allowable levels in foods for countries that report higher consumption rates of seafood, including the Maldives, Cambodia, Malaysia and Thailand (Agusa et al. 2007; FAO 1999; Harrison & Pearce 2000).

CHAPTER 4

MONITORING OF TRACE ELEMENTS AT THE LYTTELTON HARBOUR SEA-FILL, NEW ZEALAND

4.1 INTRODUCTION

Sea-fill activities with building materials can release trace elements to the surrounding environment. The immersion of construction and related materials in seawater facilitates a variety of physicochemical processes that result in the leaching of trace elements. After the Canterbury Earthquake in 2011, Lyttelton Port of Christchurch (LPC) Ltd. was granted approval by Environment Canterbury (ECan) and the Christchurch City Council (CCC) to use “clean” earthquake rubble to reclaim up to ten hectares of land in Lyttelton Harbour in Te Awaparahi Bay (LPC 2014). This rubble material included stone, bricks, tiles, aggregates, reinforced and unreinforced concrete, general rubble, glass and cured asphalt (LPC 2011). Cured asphalt was only allowed to be placed out of the wave erosion zone. Although the fill

materials were supposed to be clean there were a number of other materials inadvertently incorporated with the “clean” fill. These included treated timbers, concrete reinforced metal bars, paint chips, plastics, electrical ducting, cable sheathing and panel products. Such items, in addition to the “clean” materials, are known to release trace elements to the surrounding environment (Akter et al. 2005; Denton et al. 1997; Heck et al. 1994; Leonard 1991). Trace elements leaching out from sea-fill or coastal fill activities can enter into the marine environment leading to enhanced local levels of contamination (Denton & Morrison 2009; Jones 2010; Kjeldsen et al. 2002; Maata & Singh 2008). Release of trace elements to the surrounding ecosystem can have potentially serious implications for ecosystem and human health (Ip et al. 2007; Leivuori et al. 2000; Nfon et al. 2009; Phillips et al. 1982; Pope et al. 2011). Therefore, it is important to establish monitoring programs for quantifying trace element levels in the environments surrounding sources of potential contamination, such as the sea-fill site in Lyttelton Harbour of New Zealand.

Monitoring of trace element contamination in aquatic systems can be conducted by analysing seawater, marine sediment and biota (Rainbow 1995). The most effective monitoring programmes are those that combine multiple measures. For example, metal concentrations in seawater do not provide a complete picture of the bioavailable fraction of trace elements due to factors including the variability of water flow, tides, waves and periodicity of contaminant input (Cabral-Oliveira et al. 2015; Campanella et al. 2001). Sediments are considered a sink for contaminants released to the marine environment (Angelidis 1995), and sediment analysis therefore provides useful information about pollution in the marine environment (Calace et al. 2005). Measuring trace element concentrations in environmental matrices such as seawater or marine sediments provides information on the total contaminant load rather than the fraction that is of direct ecotoxicological relevance. As a result, the use of bioindicator or biomonitoring species is considered an important factor for assessing the bioavailable fraction of trace elements in the marine environment (Phillips 1979; Rainbow 1995; Rainbow & Phillips 1993). The use of biomonitoring species eliminates the need for complex studies on the chemical speciation and hence presumptive bioavailability of aquatic contaminants. Among the biomonitors, bivalve molluscs such as mussels have been widely used for monitoring trace elements in marine environments (Boening 1999; Chandurvelan et al. 2015; Milne 2006; Pan &

Wang 2009), as these sessile filter feeders accumulate trace elements from the environment via seawater, sediment, and food (Chan et al. 1986; Rainbow 1995; Wang & Rainbow 2008). Furthermore, mussels do not regulate toxic trace elements in their bodies, which leads to accumulation, and thus mussel tissue burdens often reflect the concentrations in the surrounding environment (Rainbow & Phillips 1993; Reinfelder et al. 1998).

Seawater, marine sediment and mussels were used to investigate trace element concentrations at the sea-fill site of Lyttelton Harbour (LH), while the sea-fill was still being created. These same matrices were concurrently examined at a reference site (Pigeon Bay; PG). The specific objectives of this study were to:

- Determine if trace elements were leaching from the sea-fill
- Determine if trace elements were accumulating in sediments due to the sea-fill
- Determine if trace element concentrations were increasing in bivalve shellfish at the sea-fill site

4.2 MATERIALS AND METHODS

4.2.1 STUDY SITE

The sea-fill site at Te Awaparahi Bay is situated between the Cashin Quay breakwater and Battery Point (BP) of Lyttelton Harbour of New Zealand. The water in this area is about 6-8 m deep with largely uniform seafloor sediment (Sneddon & Barter 2009). The seabed sediments are formed mainly from loess soils that have eroded from the surrounding hillsides (Sneddon & Barter 2009), and thus consist of sandy mud and gravel eventually leading to soft mud 50-80 m from the existing shoreline (Sneddon & Barter 2009). The harbour shore is predominantly rocky and can be described as a high energy shore (Sneddon & Barter 2009). As such it is subjected to a continual state of disturbance including elevated levels of turbidity due to high sediment mobility, generated by swells and high-wave conditions in the harbour (LPC 2009; Sneddon & Barter 2009). The mean tidal velocity at Lyttelton Harbour is 0.22 m per second, and large scale tidal circulation “gyres” exist in the

eastern half of the harbour, which turn water clockwise on ebb and counter-clockwise on flood tides (LPC 2009). The velocities during flood and ebb are constantly westward along the shore and southward along the Cashin Quay breakwater due to the clockwise eddy formed on ebb tide behind the breakwater (Inglis et al. 2006; LPC 2009).

Lyttelton Port is located adjacent to the sea-fill site (Figure 4.1), and the port handles a wide range of cargoes including coals for export, shipping vessels and, cruising vessels. As such it is a busy site with variety of anthropogenic activities (Inglis et al. 2006). Sources of contamination into Lyttelton Harbour, include sewage outfalls from the adjacent towns, storm water runoff associated with industrial sources, waste water from port operation activities, waste water from the coal stockpile yard, dry dock discharge from vessel hull maintenance, discharges from vessels, waste from engineering workshops, antifouling materials from ship hulls that stop at the port, the operation of an incinerator near Battery Point, chemicals from laboratory discharges, and previous reclamation and dredging activities (Johnston 2005; Sneddon 2011; Sneddon & Barter 2009; Sneddon et al. 2010). Lyttelton Harbour is used for many recreational activities including boating, swimming, windsurfing and recreational fishing (Boffa Miskell 2009). The sea-fill that was created beside the Lyttelton Port could be another potential source of trace elements to the harbour.

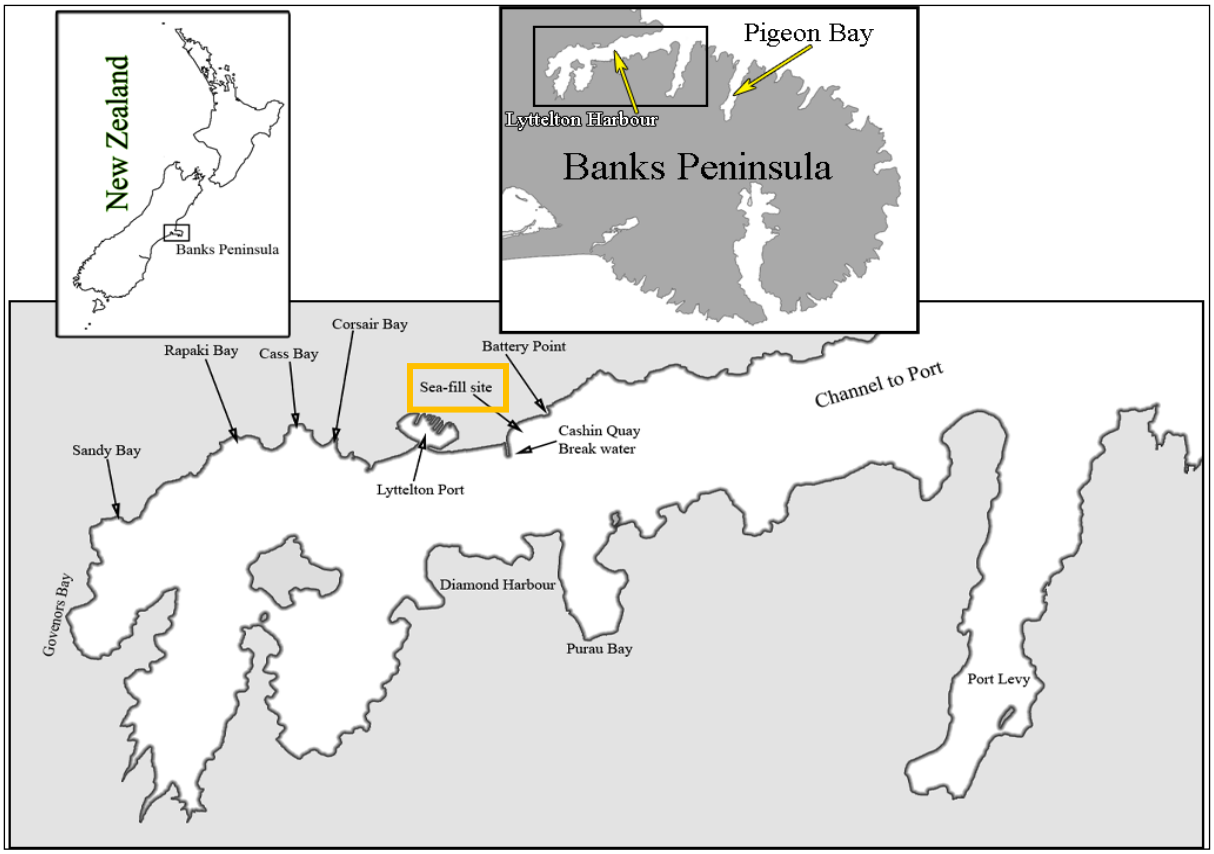


Figure 4.1: Location and map of Lyttelton Harbour and the sea-fill site, Map adapted from Google Earth

4.2.2 SAMPLE COLLECTION AND PRE-TREATMENT

4.2.2.1 SEAWATER

Sampling sites are detailed in Figures 4.2A and 4.2B. Seawater sampling was carried out quarterly, with samples taken in April 2012 (R1), July 2012 (R2), October 2012 (R3), January 2013 (R4), April 2013 (R5), July 2013 (R6), October 2013 (R7) and January 2014 (R8) along three transect lines (T1, T2, T3) at the sea-fill (Figure 4.2A), and one transect line (T4) at the reference site (Pigeon Bay) (Figure 4.2A). Pigeon Bay is a small and hydrologically separate marine inlet south of Lyttelton Harbour in Banks Peninsula. Surface seawater samples were collected by boat in an acid-cleaned plastic jug tied on an untreated wooden pole of 1.5 m long, and transferred into duplicate acid-cleaned 50 mL plastic vials. Seawater samples were also collected from each sampling point for recording pH. One field blank (50 mL Milli-Q water) for each transect line was included. Samples were taken at 2 m (A), 20

m (B), 80 m (C), 160 m (D) and 400 m (E) from the silt curtain at the sea-fill site. The silt curtain was placed at the sea-fill as a regulatory control measure to mitigate migration of sediment materials into the surrounding water. Seawater samples were kept on ice and transported to the laboratory in insulated bins. Upon return to the laboratory, seawater samples for trace element analysis were acidified to pH ≤ 2 with ultrapure HNO_3, and stored at 4°C until extracted, generally within 48 hours of sample collection. The pH of the unacidified seawater samples was also recorded upon arrival at the laboratory.

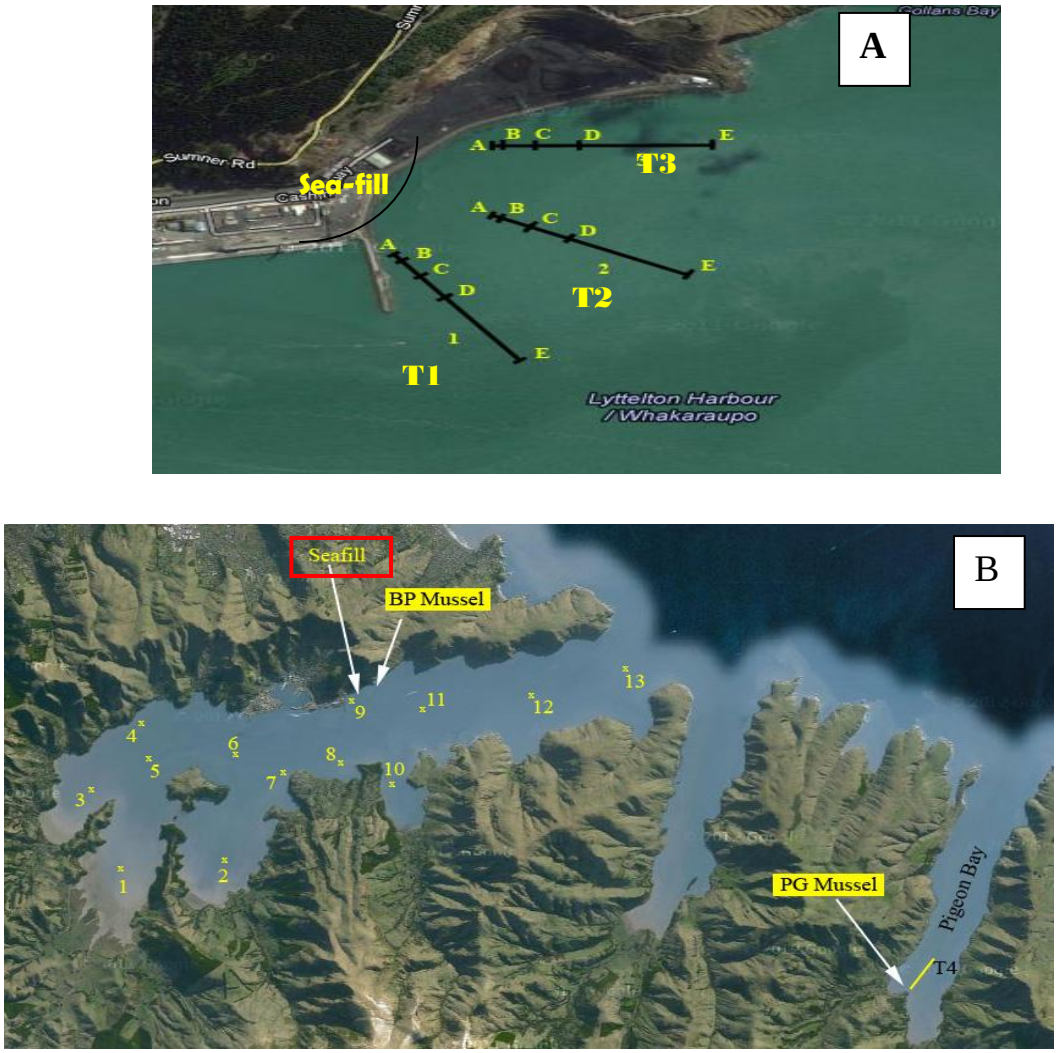


Figure 4.2: Maps showing the sampling sites in Banks Peninsula. Green mussels were collected from Battery Point (BP) and Pigeon Bay (PG). Numbers 1-13, represents sediments and seawater sites around Lyttelton Harbour- LH (map A). Transect lines at the sea-fill of LH (T 1-3) and PG (T4), and A-E on the transects represents selected distances along each transect lines from the silt curtain (map B). Maps adapted from Google Earth

4.2.2.2 MARINE SEDIMENTS

Marine sediment samples were collected every six months (April 2012 –R1, October 2012- R3, April 2013- R5 and October 2013- R7) along the same transect lines as seawater, using a stainless steel ponar grab sampler (16.5 cm × 15 cm). The sediments were transferred to acid-cleaned plastic jars with an acid-cleaned plastic spoon. The sediment samples were always collected after the seawater samples to avoid contaminating the seawater samples. Sediment samples from 13 different locations covering the entire Lyttelton Harbour (Figure 4.2A) were also collected at the beginning of the sampling work in April 2012 to determine trace element concentrations around the harbour.

Sediment samples were kept on ice and transported to the laboratory, where they were kept at 4°C until dried in an oven at 30°C for 7 to 10 days in aluminium trays. Details of sample preparation for trace element analysis is provided in Section 2.4.1.1. The distance to the sampling points from the silt curtain, the GPS coordinates of seawater and sediments, and depths of sediments at low tide are provided in Appendix B1.

4.2.2.3 GREEN-LIPPED MUSSELS

Green-lipped mussels (*Perna canaliculus*, n = 10, length 80 - 107 mm) were collected quarterly from Battery Point (adjacent to the sea-fill site). Green-lipped mussels from the PG (reference) site were collected from a site topographically similar to Battery Point (BP), that was a projection from a hill into the sea adjacent to the transect line T4 (Figure 4.2A). The green-lipped mussels were collected at the same time as seawater. Green-lipped mussels were not able to be collected in the sampling round R3 (October 2012) due to bad weather. Green-lipped mussels were kept on ice during transport to the laboratory where they were kept at 4°C until processed the next day. The shells were cleaned of any epibionts, whole body weights were recorded, and the mussels dissected. Whole soft tissue was then collected; and after wet weights were recorded, tissues were stored at -20°C until freeze-dried for analysis.

4.2.3 DETERMINATION OF TOTAL ORGANIC CARBON IN SEDIMENTS

Marine sediments (< 2 mm) in sampling rounds R1 and R2 from the four transect lines (T1-4) were analysed for total organic carbon by loss on ignition (LOI). Approximately 3 g of each sediment were accurately weighed into silica crucibles and heated in a muffle furnace at 500°C for four hours. Samples were cooled to approximately 150°C before being removed from the furnace, and left to cool further in a desiccator. Once cold (room temperature), the samples were reweighed and the percentage mass difference calculated to provide a measure of organic content.

4.2.4 SAMPLE PREPARATIONS FOR TRACE ELEMENT ANALYSIS BY ICP-MS

The laboratory experimental methods to extract trace elements in seawater, marine sediments and green-lipped mussels, and their preparation for chemical analysis by ICP-MS, are provided in Chapter 2 (Section 2.3 and 2.4).

4.2.5 METAL POLLUTION INDEX (MPI)

Metal pollution index (MPI) values were calculated for seawater, marine sediments and green mussels. These values were used to compare trace element levels at the sea-fill and the reference site. The MPI values were calculated using Equation 4.1 (Usero et al. 2005).

$$MPI = (Cf_1 \times Cf_2 \dots Cf_n)^{1/n} \qquad \textbf{Equation 4.1}$$

where Cf is the concentration of the trace element (dry weight), and n is the number of trace elements analysed.

4.2.6 STATISTICAL ANALYSIS

All statistical analyses were carried out in R© (Version 2.15.3). Statistical analysis was only performed for sample sets where more than 50% of the samples had values above the limit of quantification (LOQ). In conditions where more than 50% of the samples were above the LOQ, the remaining samples below LOQ were given a value of half the LOQ. All duplicate measurements were averaged before inclusion in the statistical analysis.

All data were checked for normality by plotting probability plots. Where necessary, data were log transformed to meet assumptions of normality before analysis. Significant differences ($p < 0.05$) at the 95% confidence level for trace element concentrations in the environmental samples between the sites, between the transects, variations with distance from silt curtain (sampling point) and variations with respect to time (sampling rounds) were determined by using multifactor ANOVA tests followed by Tukeys HSD tests, at $\alpha = 0.05$. Pearson's correlation coefficients were also used to analyse relationships between trace elements within and between the environmental matrices.

4.3 RESULTS

4.3.1 ANALYTICAL METHOD PERFORMANCE

Standard reference material of mussel tissue (SRM-2976-EVISA) and fish protein (DORM-3, NRCC) were digested along with the green-lipped mussels. Standard reference marine sediment (SRM-2702-NIST) was digested along with the marine sediments. Certified reference seawater (NASS-6, NRCC) and trace element-spiked water samples were extracted with the seawater samples.

Percentage recoveries of cadmium, copper, iron, lead, yttrium and zinc in certified reference seawater (NASS-6), and trace element-spiked Milli-Q water, natural seawater and artificial seawater were within acceptable ranges (85-120%) (Table B2.1, Appendix B2). The mean percentage recoveries of all elements in the standard reference mussel, fish protein (DORM-3) and marine sediments ranged between 90 and 113% with the exception of lead in DORM-3 (35.7%) (Table B2.2, Appendix B2).

4.3.2 TRACE ELEMENTS IN SEAWATER

The trace element concentrations (mean values ± SE in μg L^{-1}) in seawater along the transect lines are presented in Appendix B2.3, and the concentrations of trace elements in the seawater collected around the harbour are presented in Appendix B3. The graphical comparisons of variations in trace element concentrations with

respect to time, site, and distance from the silt curtains are presented in Figure 4.3a-e. The trace elements analysed in seawater were cadmium, copper, iron, lead and zinc (mercury and arsenic were not able to be analysed by the employed extraction method). The pH values of the seawater samples ranged from 7.74 to 8.16 for both the sea-fill site and the reference site throughout the sampling period, and were not significantly different between the two sampling sites.

In general, the concentrations of trace elements in the seawater at the sea-fill site were higher than those of the reference site (PG). Concentrations of seawater cadmium, copper, iron, lead and zinc are presented in Figures 4.3a-e respectively. Concentrations of cadmium, lead and zinc were higher closer to the sea-fill and decreased with distance from the sea-fill. Copper and iron did not show any significant variations with respect to distance from the sea-fill. Although there was no specific pattern of variation between the sampling times, levels of trace elements tended to be higher in the first sampling rounds than in later sampling rounds.

More specifically, cadmium concentrations in seawater of T1, T2, T3 (sea-fill) were significantly higher than T4 (PG) (also T1 > T3 at the sea-fill site). Cadmium concentrations in sampling rounds R1, R2 and R3 were greater than R5, R6, R7 and R8 at the sea-fill site, with the reference site showing similar results where R1 exhibited higher cadmium levels than all other sampling times except R4. The combined results of all 8 sampling times indicated that the cadmium concentrations in the seawater at 2 m from the silt curtain at the sea-fill were significantly higher than at 160 m and 400 m, reflecting a decrease in concentration away from the sea-fill site.

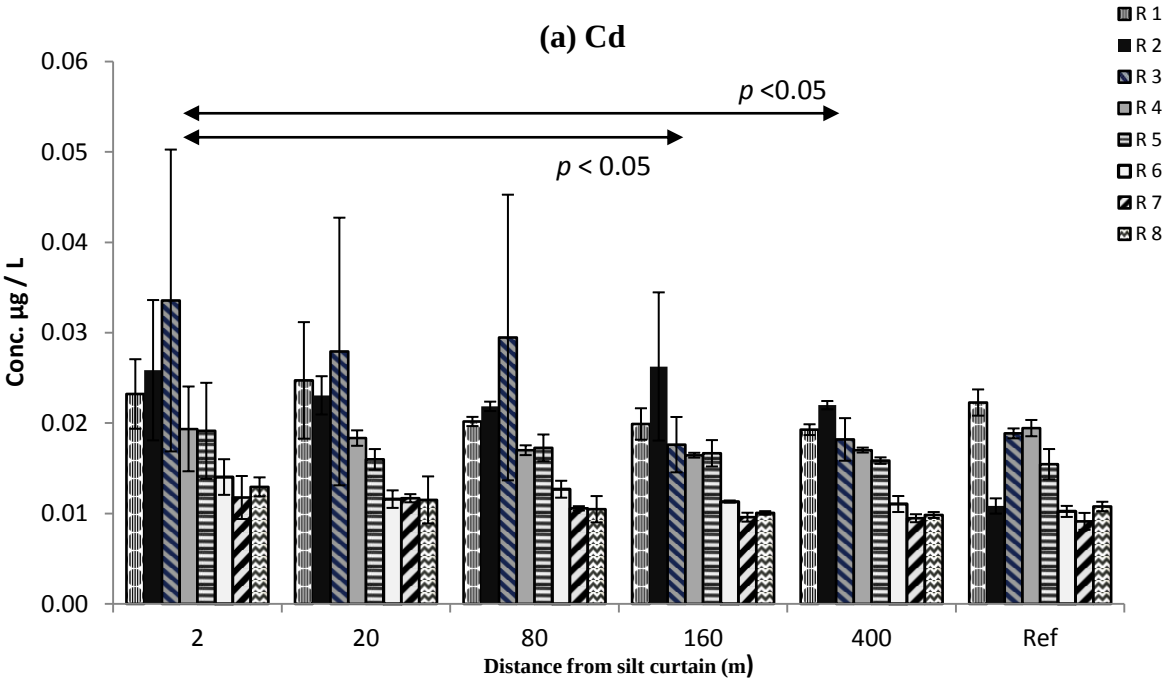
Concentrations of copper at transect lines T1, T2 and T3 were significantly higher than T4 (reference site), with no difference between the three transect lines at the sea-fill site. Copper concentrations in transects over time followed the general pattern seen for cadmium with R1 significantly elevated relative to all later sample times (except R2).

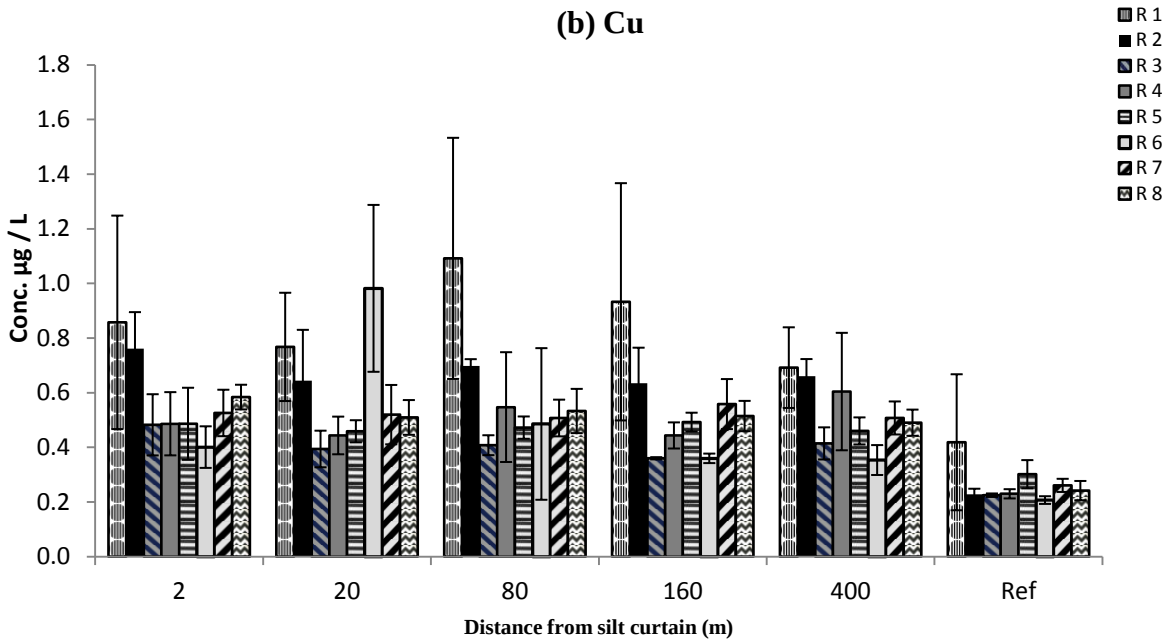
The iron concentrations at transect line T2 were significantly higher than those at T4. In general, the iron concentrations in the sampling rounds R1, R2 and R4 were significantly higher than all other sampling rounds at the sea-fill site, and R6 and R8 were lower than all other sampling rounds. At the reference site, iron concentrations

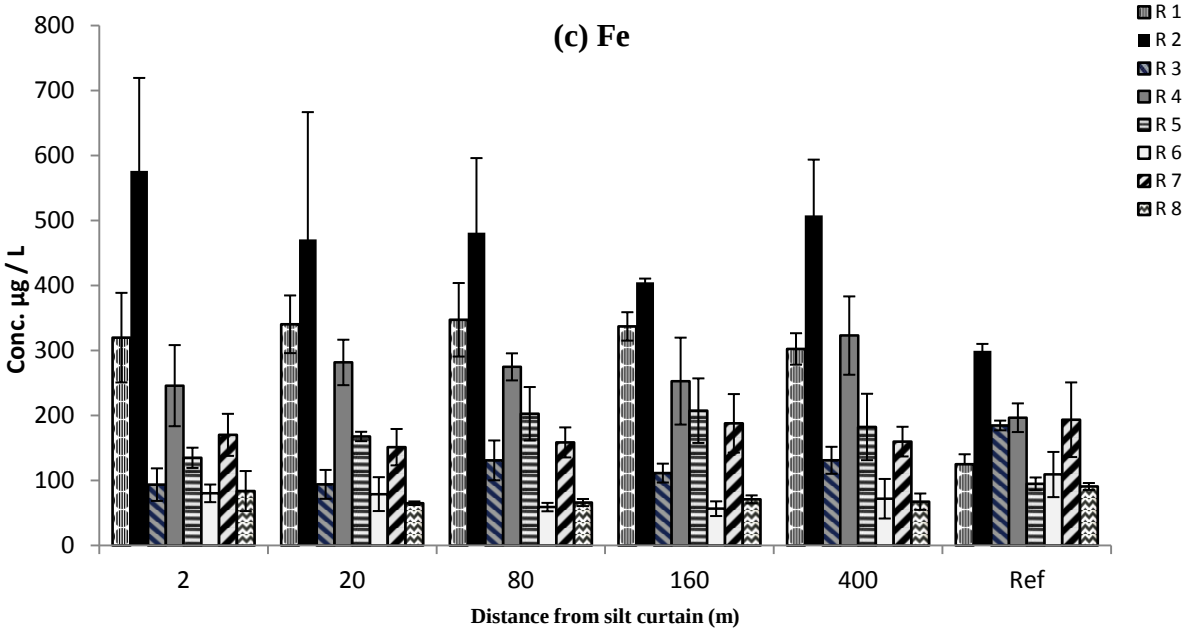
in sampling round R2 were significantly higher than in all other sampling rounds.

Lead concentrations were significantly higher in all sea-fill transects (T1, T2 and T3) than in the reference transect (T4). The lead concentrations at 2 m from the silt curtain were significantly higher than lead in samples taken at distances 80, 160 and 400 m from the silt curtain, indicating concentrations decreased away from the sea-fill. Zinc followed a similar pattern, with higher levels in the sea-fill transects, and significantly decreasing zinc levels with distance along the sea-fill transects.

The correlation analysis of trace elements within seawater (Table B4.1, in Appendix B4) indicated all elements at the sea-fill site were strongly positively correlated to each other with the exception of cadmium and lead, which were not correlated. At the reference site, iron was significantly positively correlated to lead and zinc, whereas copper was significantly positively correlated to cadmium and lead, and lead was significantly positively correlated to cadmium.




(b) Cu
R 1
R 2
R 3
R 4
R 5
R 6
R 7
R 8
Conc. µg / L
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2
20
80
160
400
Ref
Distance from silt curtain (m)


(c) Fe
R 1
R 2
R 3
R 4
R 5
R 6
R 7
R 8
Conc. µg / L
800
700
600
500
400
300
200
100
0
2
20
80
160
400
Ref
Distance from silt curtain (m)

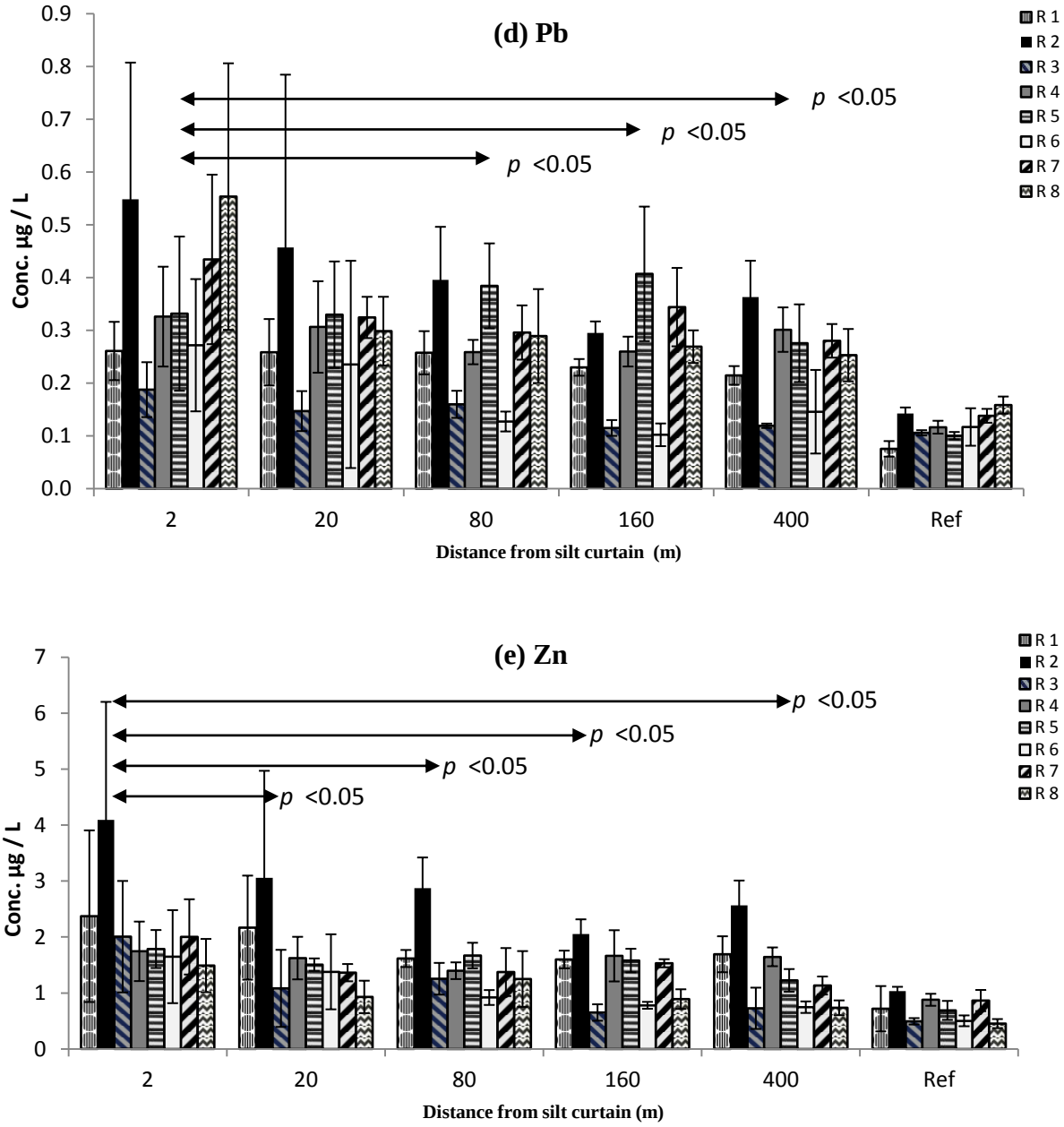


Figure 4.3 (a-e): Comparison of mean trace element concentrations in seawater with distance from silt curtain at the sea-fill sites as a function of sampling round. Plotted values represent means ± standard errors (n = 3). Significant differences (p < 0.05) in trace element concentration with respect to distance are indicated with the arrows.

4.3.3 TRACE ELEMENTS IN MARINE SEDIMENTS

The trace element concentrations (mean values ± SE in µg g dry wt^{-1}) in marine sediments collected along the transect lines are presented in Appendix B5, and the graphical comparisons of the trace element concentrations with respect to distance

from the silt curtain, time (sampling rounds) and sites are presented in Figure 4.4. Trace element concentrations in the sediments of Lyttelton Harbour are presented in Appendix B3 along with seawater results. The trace elements analysed in marine sediments were arsenic, cadmium, copper, iron, lead and zinc.

Overall, sediment results indicated that cadmium, copper, iron, lead and zinc concentrations were significantly higher at the sea-fill site compared to the reference site. Arsenic concentrations at the sea-fill site were not significantly different from the reference site, and there were no significant differences between any transect lines (T1= T4 = T3 = T4). Arsenic, cadmium and lead concentrations were higher closer to the silt curtain and decreased away from the sea-fill (T1, T2 and T3 combined), while other elements did not vary significantly with respect to distance. Arsenic and iron concentrations increased with respect to time at the sea-fill site, while copper decreased with respect to time, and other elements did not change significantly over time.

The overall arsenic concentrations varied with time at the sea-fill site, with R3, R5, R7 > R1, and R7 > R3. At the reference site, the arsenic concentrations also varied in the following manner: R7 = R5 > R3 = R1. The arsenic concentrations also varied with distance from the silt curtain, with samples at distances of 2 m and 20 m significantly higher than samples collected at 400 m. Arsenic concentrations increased with respect to time at the sea-fill as well as at the reference site over the sampling period.

Cadmium concentrations in T1, T2, T3 (sea-fill) > T4 (PG); while within sea-fill site transects, T1 and T3 > T2. There were no significant differences with respect to time at either sampling site. Cadmium concentrations were significantly higher at distance of 2 m from the silt curtain than at 400 m at the sea-fill (T1, T2 and T3 combined).

Copper concentrations in T1, T2 and T3 > T4 (PG); while T1 > T2 and T3 at the sea-fill site. Copper concentrations in R1 and R3 > R7 at the sea-fill site, while there were no significant differences with respect to sampling time at the reference site. Although the copper concentrations decreased with respect to time at the sea-fill, there were no significant differences with respect to distance.

Iron was significantly higher in sediments collected from the sea-fill site than those sourced from the reference site. Iron levels in sediments also increased with time, with R7 showing an elevated level with respect to R1 and R3 at the sea-fill site. Similar findings were observed at the reference site with respect to sampling round. There was no significant effect of distance from the silt curtain on iron sediment concentrations.

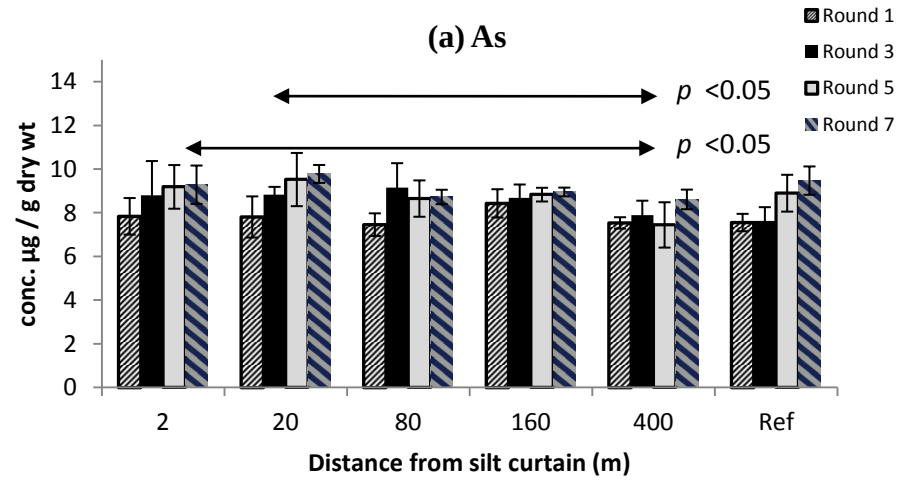
Lead concentrations at T1, T2 and T3 > T4; while T1 > T2. Although there were no significant differences in sediment lead with respect to time at the sea-fill site or the reference site, the concentrations at 2 m from the silt curtain were significantly higher than at distances 160 m and 400 m from the silt curtain.

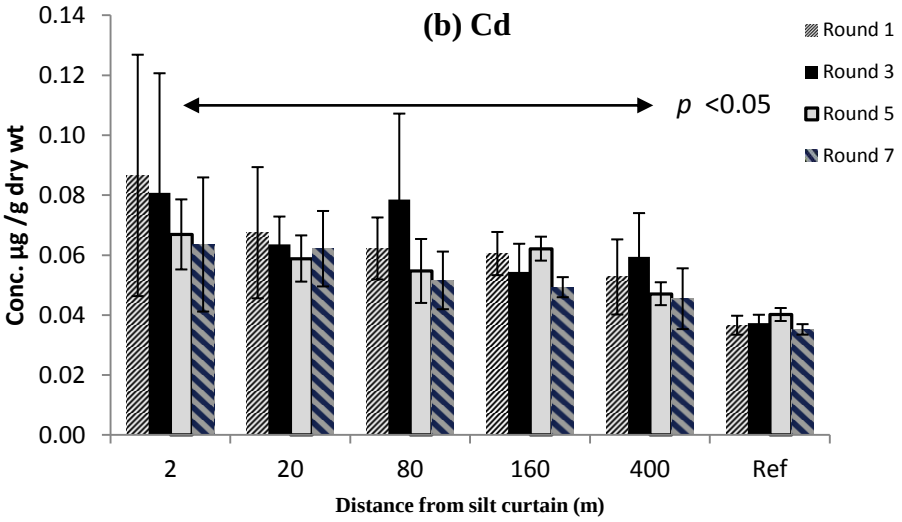
Concentrations of zinc at the transect lines T1, T2 and T3 > T4; while T1 > T2 and T3. There were no significant differences in sediment zinc between the sampling rounds at either site, and no significant differences with respect to distance at the sea-fill site.

The correlation analysis of trace element levels within the sediments (Table B4.2 in Appendix B4) of the sea-fill indicated that iron was significantly negatively correlated with cadmium, lead and zinc while arsenic was significantly positively correlated to iron. Copper was significantly positively correlated with cadmium, lead and zinc; while zinc was significantly positively correlated to cadmium and lead. Finally, cadmium was significantly positively correlated with lead at the sea-fill site.

At the reference site iron was significantly positively correlated to arsenic, copper, lead and zinc. Copper was significantly positively correlated to arsenic, lead and zinc; while zinc was significantly positively correlated to arsenic and lead. Lead was also significantly positively correlated to arsenic.

Analysis of trace elements that were correlated between seawater and sediment samples (Table B4.3 in Appendix B4) indicated that sediment copper was significantly positively correlated to seawater copper, and sediment zinc correlated with seawater zinc at the sea-fill site. There were no significant correlations at the reference site.


(a) As
Round 1
Round 3
Round 5
Round 7
p <0.05
p <0.05
conc. µg / g dry wt
14
12
10
8
6
4
2
0
2
20
80
160
400
Ref
Distance from silt curtain (m)


(b) Cd
Round 1
Round 3
Round 5
Round 7
p <0.05
Conc. µg /g dry wt
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
2
20
80
160
400
Ref
Distance from silt curtain (m)

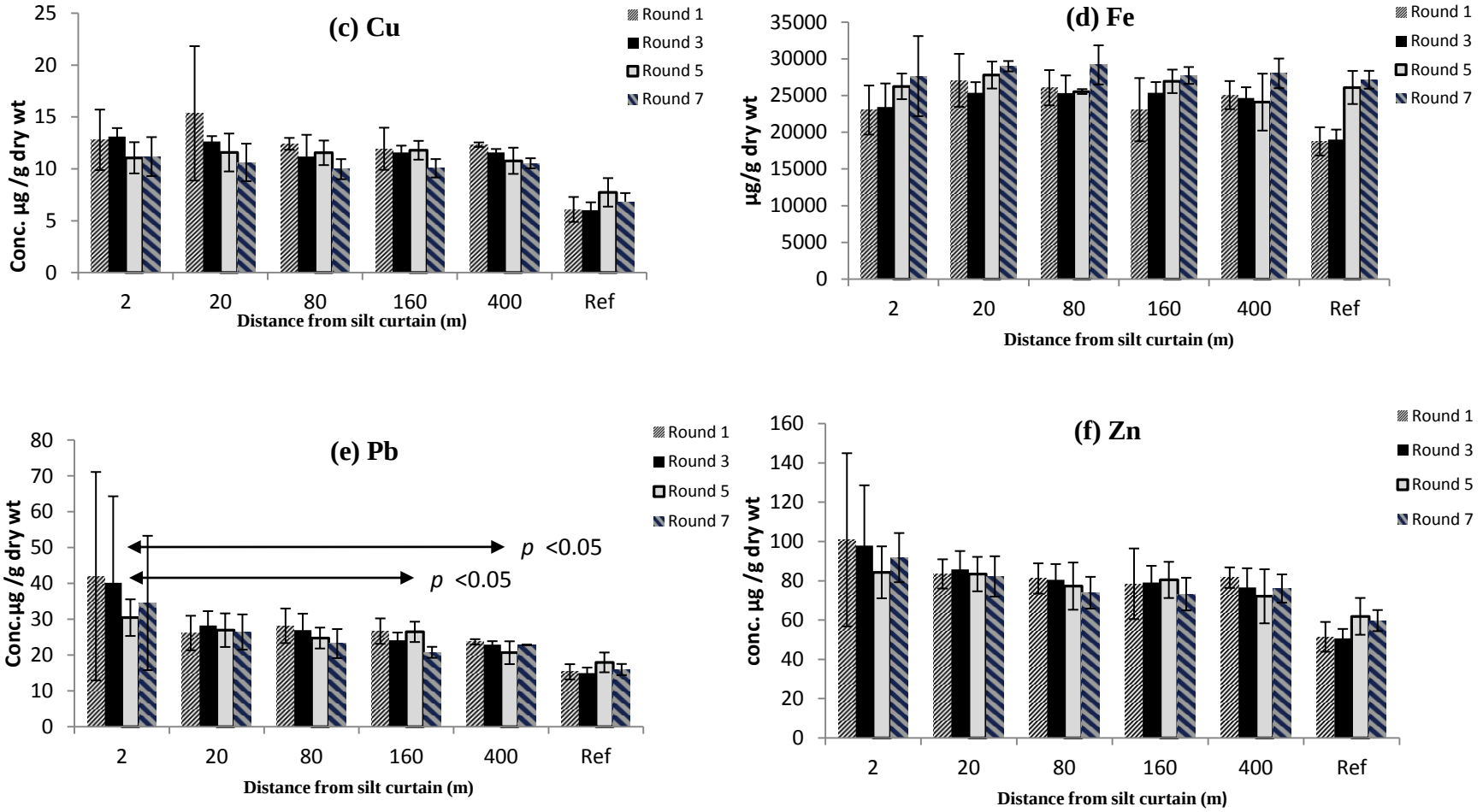


Figure 4.4 (a-f): Comparison of mean trace element concentrations in sediments along the transect lines at the sea-fill site, and Pigeon Bay (PG) as a function of distance from the silt curtain and sampling round. Plotted values represent means ± standard errors (n = 3). Significant differences with respect to distance along the transect lines (sea-fill site) are indicated with the arrows.

4.3.3.1 ORGANIC CONTENT OF THE SEDIMENTS

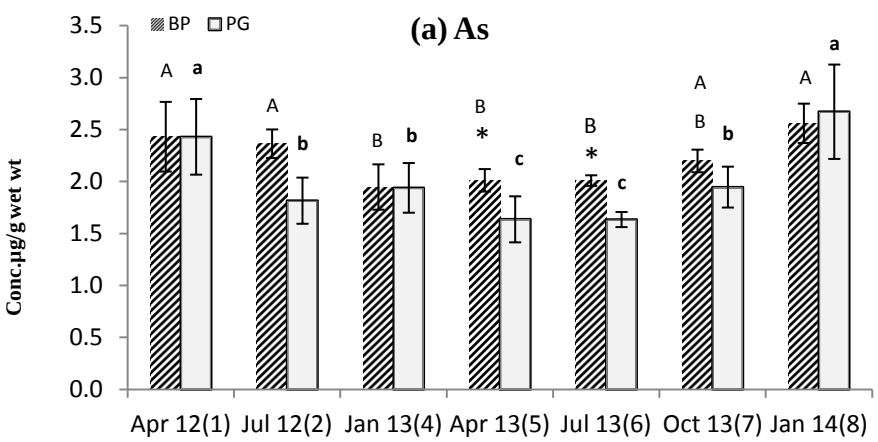
The percentage of organic content (Table B6 in Appendices) in the sediments of the first two sampling rounds ranged from 1.35 to 11.5% at the sea-fill site and from 3.8 to 7.1% at the reference site (PG). There were no significant differences in organic content with respect to distance, transect lines or time at the sea-fill. However, organic content at the reference site was greater in the sampling round R3, and was elevated relatively to that at R1 at reference site. Copper concentrations were significantly ($p < 0.05$) positively correlated with the organic content at the sea-fill site, while arsenic, cadmium and iron were significantly positively correlated with the organic content at the reference site.

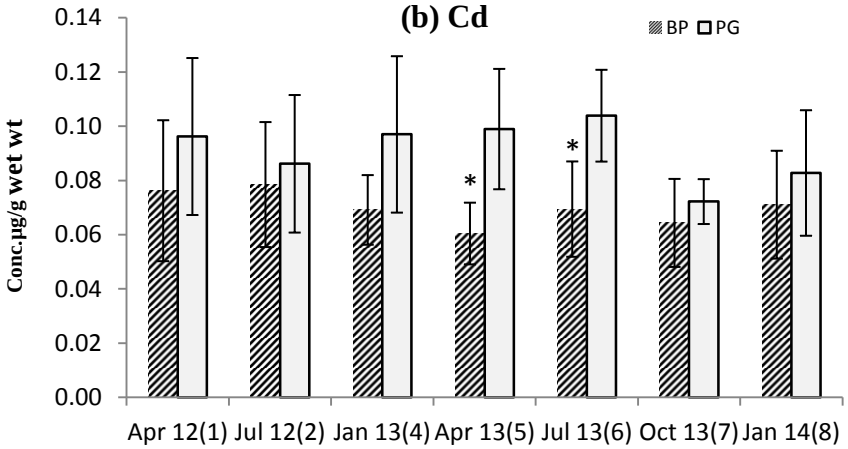
4.3.4 TRACE ELEMENTS IN GREEN-LIPPED MUSSELS

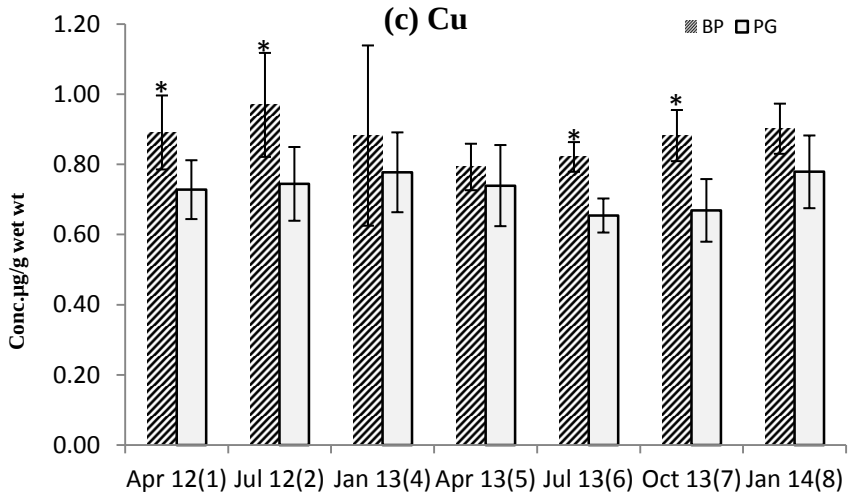
The trace element concentrations (mean values ± SE in µg g wet wt^{-1}) in green-lipped mussels during the two years of sampling are presented in Appendix B7, and the graphical comparisons of the trace element concentrations with respect to time and site are presented in Figure 4.5a-g. Comparisons of concentrations of arsenic, cadmium, copper, iron, mercury, lead and zinc in green-lipped mussels are presented in Figures 4.5a-g respectively.

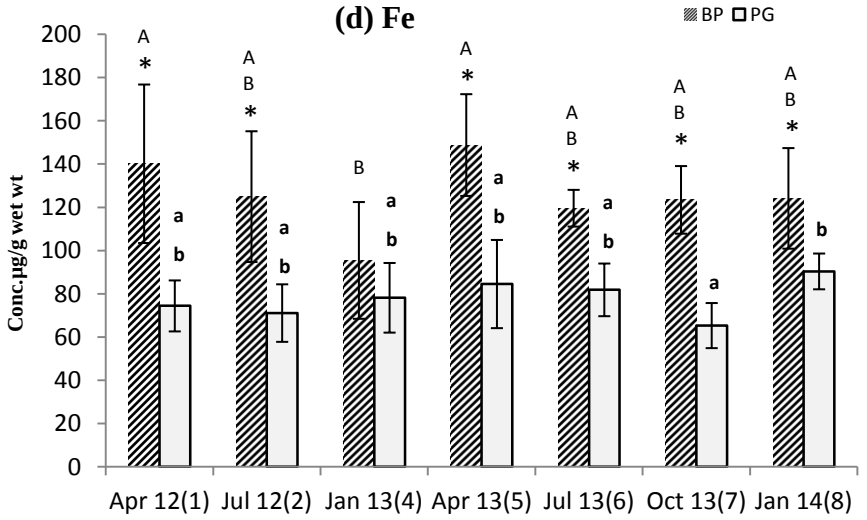
Overall, arsenic, copper, iron, mercury, lead and zinc concentrations were higher in the samples collected from the sea-fill, while cadmium was higher in the samples from PG. In general, the trace element concentrations in green-lipped mussels showed variations with time in a manner that suggested changes related to season. The cadmium concentrations in the green-lipped mussels of PG were significantly higher in the sampling rounds R5 and R6 compared to those of sea-fill samples, but there were no differences with respect to sampling time within any one sampling site. Mercury concentrations in the green-lipped mussels from the sea-fill (BP) were significantly higher than in the PG samples for all the sampling rounds with the exception of sampling round R5, and the mercury concentrations in the samples of R8 at the PG site were below LOQ. Lead concentrations in the green-lipped mussels from the sea-fill were significantly higher than those of PG throughout the sampling period. Significant differences with respect to sampling site, and time within individual sites, are shown in Figures 4.5a-g. The correlation analysis of trace

elements in green-lipped mussels and seawater did not show any significant relationships at the sea-fill site or the reference site (Appendix B8). Similarly there were no significant correlations between trace elements in sediments and mussels at either site (Appendix B9).


(a) As
BP PG
Conc.µg/g wet wt
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
A a
A b
B b
B * c
B * c
A B b
A a
Apr 12(1) Jul 12(2) Jan 13(4) Apr 13(5) Jul 13(6) Oct 13(7) Jan 14(8)


(b) Cd
BP PG
Conc.µg/g wet wt
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
*
*
Apr 12(1) Jul 12(2) Jan 13(4) Apr 13(5) Jul 13(6) Oct 13(7) Jan 14(8)


(c) Cu
BP PG
Conc.µg/g wet wt
1.20
1.00
0.80
0.60
0.40
0.20
0.00
*
*
*
*
Apr 12(1) Jul 12(2) Jan 13(4) Apr 13(5) Jul 13(6) Oct 13(7) Jan 14(8)


(d) Fe
BP PG
Conc.µg/g wet wt
200
180
160
140
120
100
80
60
40
20
0
A * a b
A B * a b
B a b
A * a b
A B * a b
A B * a
A B * b
Apr 12(1) Jul 12(2) Jan 13(4) Apr 13(5) Jul 13(6) Oct 13(7) Jan 14(8)

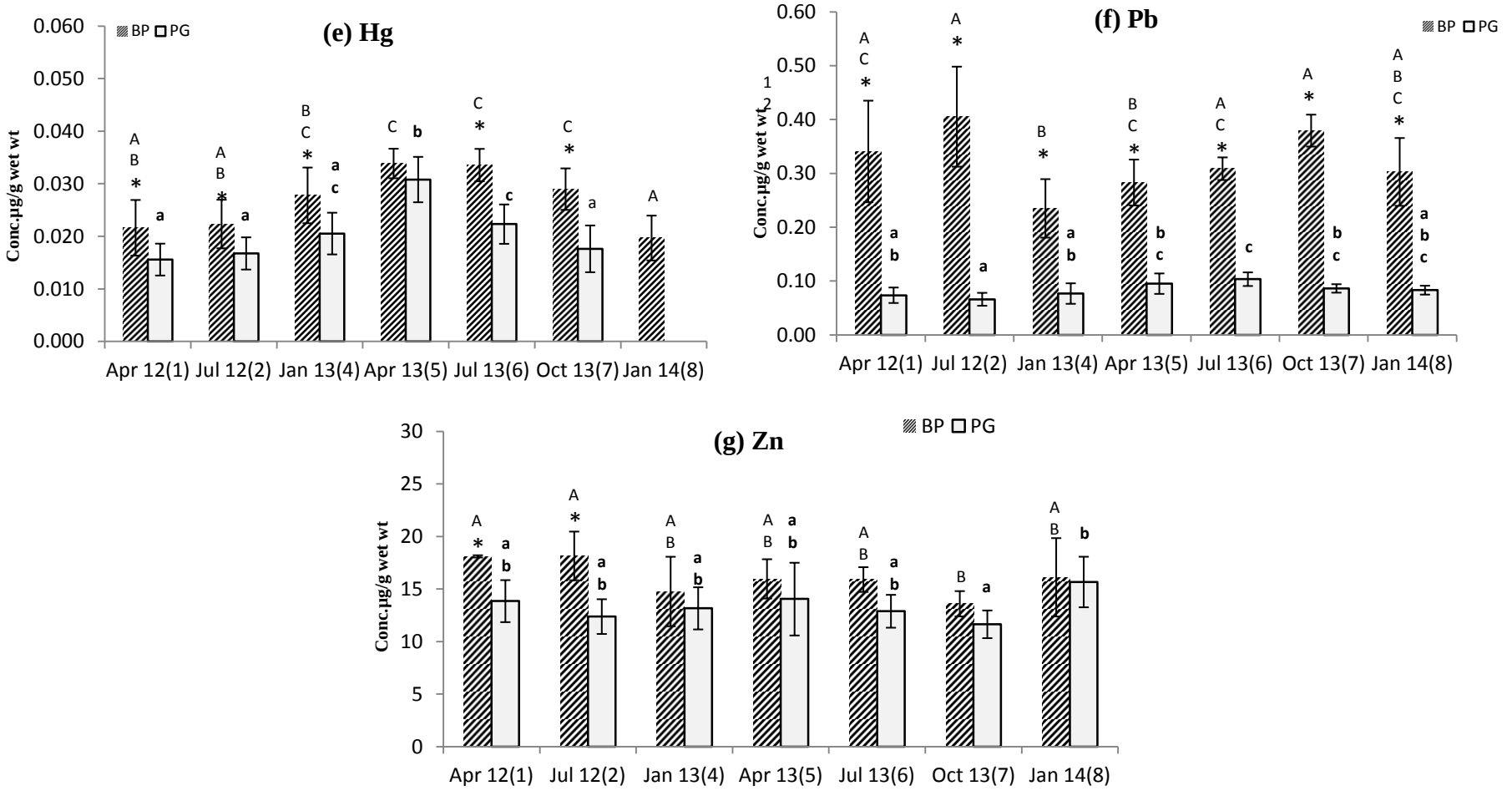


Figure 4.5 (a-g): Comparison of mean trace element concentrations in green-lipped mussels from sea-fill and reference (PG) over time (green-lipped mussels were not collected in 3rd round). Plotted values represent means ± standard errors (n = 10). The asterisks indicate significant differences between the sites within a sampling time. Bars sharing uppercase letters are not significantly different with respect to sample time within the sea-fill site, and bars sharing lowercase letters are not significantly different with respect to sampling times within the reference site (PG). Mercury was <LOQ in green mussels in sampling round 8 at PG. (Note: BP- a site in the vicinity of the sea-fill)

4.3.5 METAL POLLUTION INDEX (MPI), AND GUIDELINE VALUES

Metal pollution index (MPI) values derived from the seawater, sediment and green-lipped mussel trace element concentrations for the two sampling sites are presented in Tables 4.1 - 4.3 respectively. Trace element concentrations obtained in the current study for seawater, sediment and green-lipped mussel tissue (whole tissue) are also summarised in Tables 4.1 - 4.3, along with regulatory values and values for similar studies in the literature.

When based on seawater and marine sediment results, transect line T1 generated the highest MPI value, followed by T2 and T3. Using the same matrices, the lowest MPI values were obtained for T4 at the reference site (Pigeon Bay) (Table 4.1 and 4.2). Similarly, the MPI values obtained using the trace element content in the green-lipped mussels also showed that the reference site contained the lowest MPI value (Table 4.3).

Table 4.1: Mean, minimum and maximum concentrations (µg L^{-1}) for trace elements in seawater, ANZECC guideline values, MPI values derived for each transect line at the two sampling sites, and comparisons of results with similar studies

Sample / location	Fe	Cu	Zn	Cd	Pb	MPI
Seawater (µg L^{-1})						
T1	212 (44 - 688)	**0.66 (0.35 - 3.29**)	1.83 (0.69 - 6.10)	0.018 (0.010 - 0.036)	0.33 (0.08 - 0.55)	1.09
T2	213 (62 - 396)	**0.54 (0.30 - 0.88)**	1.55 (0.61 - 3.16)	0.018 (0.009 - 0.048)	0.27 (0.06 - 0.84)	0.98
T3	208 (61 - 699)	**0.54 (0.31 - 1.63)**	1.41 (0.48 - 4.28)	0.016 (0.009 - 0.026)	0.25 (0.11 - 0.62)	0.91
T4 (PG)-ref. site	162 (71 - 299)	0.26 (0.18 - **0.86)**	0.70 (0.35 - 1.44)	0.015 (0.009 - 0.025)	0.12 (0.07 - 0.18)	0.56
ANZECC trigger values for marine water (µg L^{-1}) for level of protection of marine species (% species)						
99% protection	**	0.30	7.00	0.70	2.20	
95% protection	**	1.30	15.00	5.50	4.40	
90% protection	**	3.00	23.00	14.00	6.60	
80% protection	**	8.00	43.00	36.00	12.00	
Comparisons with other similar studies						**Reference**
Bermuda coastal fill	<10	0.12 - 18.4	3.8 - 204	0.02 - 0.07	0.05 -1.5	(Jones 2010)
Lyttelton Harbour	NA	<1.1 - 1.2	<4.2 - 8.8	NA	<1.1 - 2.7	(Sneddon 2011)
Thilafushi Island / Maldives	9.7- 31.4	0.05-16.3	0.84-5.3	0.012-0.019	0.02-0.80	Chapter 3 of this study

**** Values not provided in the ANZECC guideline. MPI-metal pollution index. NA- trace elements not analysed**

Table 4.2: Mean, minimum and maximum concentrations (µg g dry wt^{-1}), of marine sediment, the ANZECC guideline values, and the MPI values derived for the transect lines at the two sampling sites.

Sample	Fe	Cu	Zn	As	Cd	Pb	MPI
Marine sediment (µg g dry wt^{-1})							
T1	25348 (19187 - 29738)	12.97 (11 - 22)	93.22 (77 - 152)	8.43 (7 - 9)	0.072 (0.040 - 0.133)	32.37 (20 - **75**)	29.03
T2	26760 (24623 - 28908)	10.78 (9 - 13)	78.87 (71 - 86)	8.76 (8 - 10)	0.050 (0.039 - 0.061)	22.47 (19 - 26)	24.62
T3	26004 (18199 - 32135)	11.36 (9 - 13)	73.93 (56 - 90)	8.53 (6 - 10)	0.063 (0.046 - 0.086)	27.08 (17 - 30)	26.09
T4 (PG)	22754 (16878 - 28635)	6.67 (5 - 9)	55.95 (45 - 73)	8.39 (7 - 10)	0.042 (0.033 - 0.044)	16.04 (14 - 20)	19.09
ANZECC Interim Sediment Quality Guideline (ISQG) values for the protection of marine species (µg g dry wt^{-1})							
ISQG-low	**	65	200	20	2	50	
ISQG-high	**	270	410	70	10	210	
Comparisons with other similar studies							**Reference**
Lyttelton sea-fill / NZ	NA	8 - 15	54 - 93	6 - 8	NA	22 - 49	(Sneddon 2011)
Kiribati	NA	0.3 - 14	1.2 - 77	< LOQ	0.3 - 14	3.4 - 13	(Redfern 2006)
Te Atatu Peninsula / NZ	NA	14 - 48	97 - 896	2.6 - 36	14 - 48	23 - 130	(Redfern 2006)
Suva Harbour / Fiji	NA	59 - 306	88 - 670	0.7 - 45	59 - 306	19 - 272	(Naidu & Morrison 1994)
Bermuda	800- 11100	3 - 159	16.6 - 1380	2.7 - 29	3 - 159	15 - 259	(Jones 2010)
Tanapag Lagoon / Saipan	NA	0.22 - 28	1.63 - 127	1.33-10	0.22 - 28	<0.4 - 41	(Denton et al. 2001)
Thilafushi Island / Maldives	22.56 - 13562.81	0.14 -148	0.21- 425.81	1.5-6.1	0.04 - 0.44	0.20 - 30.69	Chapter 3 of this study

**** Values not provided in the ANZECC guideline. MPI-metal pollution index. NA- trace elements not analysed.**

Table 4.3: Mean, minimum and maximum concentration (µg g wet wt^{-1}) values of green-lipped mussels, the MPI values derived from each sampling site, and the FSANZ maximum allowable level for shellfish

Trace element	Battery Point (BP)	Reference site (PG)	FSANZ maximum allowable level –ML (µg g wet wt^{-1})
Fe	125 (64 - 191)	89 (50 - 196)	**
Cu	0.88 (0.73 - 1.51)	0.73 (0.57 -0.96)	**
Zn	16.10 (10 - 22)	13.39 (10 - 19)	**
As (total)	2.22 (1.58 - 3.19)	2.01 (1.43 - 3.64)	1 (inorganic)
Cd	0.070 (0.046 - 0.135)	0.091(0.043 - 0.150)	2
Hg	0.027 (0.046 - 0.135)	0.020 (0.043 - 0.150)	0.5
Pb	0.32 (0.14 - 0.53)	0.09 (0.05 - 0.19)	2
MPI values	**1.13**	**0.84**	

** Values not provided in the FSANZ standard.

4.4 DISCUSSION

4.4.1 TRACE ELEMENTS IN SEAWATER AND SEDIMENT

Limited studies have been conducted that examine trace element concentrations in seawater of coastal landfill or sea-fill sites. In this study, the concentrations of iron were the highest amongst all the trace elements analysed in seawater, followed by zinc, copper, lead, and cadmium. The high content of iron in the samples could be because the seawater samples were not filtered prior to the analysis, and thus particulate and colloidal iron in the seawater samples may have been present. The order of trace element concentrations in this study was generally in agreement with the results of the contaminant monitoring work for reclamation activities in Lyttelton Harbour (Sneddon 2011), although iron was not analysed in this previous report. However, the lead concentrations reported by Sneddon (2011) were greater than those of this study (Table 4.2).

Previous studies on trace element concentrations in seawater of coastal fills are presented in Table 4.1 for comparison to the current data. Copper, zinc, and lead levels at the Bermuda marine fill site were comparatively higher than those of this

study (Jones 2010). Copper concentrations at Thilafushi Island of the Maldives were also significantly higher than those of the Lyttleton Harbour sea-fill (Chapter 3). These differences could be related to the nature of the fill materials. The coastal fills of Bermuda and Thilafushi Island of the Maldives contained a variety of waste, including metallic and building waste, broken boat parts, engines, cars, fridges, hazardous chemicals, municipal solid waste, and incinerator ash (CDE Consulting 2011; Jones 2010). These are potential sources of trace elements to the surrounding environment. Moreover, these coastal fill and sea-fill sites did not have control mechanisms to mitigate escape of materials into the surrounding environment, such as retaining wall linings or silt curtains like the sea-fill site of Lyttelton Harbour (CDE Consulting 2011; Jones 2010).

Similar to seawater, the highest concentration of trace elements in the sediments was iron, followed by zinc, lead, copper, arsenic, and cadmium at the sea-fill site and the sediments collected around the harbour. This order is generally in agreement with the monitoring work carried out at the site prior to the sea-fill activities (Sneddon 2011). The order also reflects the general pattern of natural crustal abundance and marine sediments at other sea-fill sites such as Thilafushi Island of Maldives (Chapter 3), the marine landfill beside a coral reef in Bermuda (Jones 2010) and other similar studied sites (Glasby et al. 1988; Williamson 1992) (Table 4.2).

Correlation analysis of the sediment trace elements indicated that cadmium, copper, lead, and zinc were significantly positively correlated, as were iron and arsenic. Conversely iron was negatively correlated to zinc, cadmium, and lead in the sea-fill sediments of this study. This finding was different from the results found for volcanic-derived sediments at Paygo Bay in Guam, Laucala Bay in Fiji, and Fanga'uta Lagoon in Tonga (Denton & Morrison 2009; Morrison & Brown 2003; Morrison et al. 2001), where the iron-copper and iron-zinc pairs were highly positively correlated. In contrast to the correlations between trace elements in the sea-fill sediments, iron at the reference site was correlated with all trace elements with the exception of cadmium. These results suggest that the input sources of cadmium, copper, lead and zinc could be different from the sources of arsenic and iron at the sea-fill site.

The MPI values at the sea-fill site compared to the reference site suggest that

the sea-fill site is a more contaminated site. The significantly higher concentrations of trace elements (cadmium, lead, and zinc in seawater; and arsenic, cadmium, and lead in sediment) closer to the silt curtain at the transect lines of the sea-fill site indicate that the sea-fill itself was a source of trace elements to the adjacent marine environment. Other studies on trace elements in marine sediments beside coastal landfills and sea-fills have also shown that fill activities contribute trace elements to the marine environment (Denton & Morrison 2009; Jones 2010; Maata & Singh 2008; Naidu & Morrison 1994). For example, previous studies of seawater trace elements at the marine landfill in Bermuda (Jones 2010), and the sea-fill of Thilafushi Island of Maldives (Chapter 3 of this thesis) showed a similar characteristic pattern of diminishing trace element concentrations with distance from the sea-fill site.

Iron concentrations in this study were higher than those of the marine landfill sites in Bermuda and Thilafushi Island (Table 4.2). The higher concentrations of iron in the LH sea-fill site and the reference site indicate that iron in the sampling area was naturally high. It is common to see elevated levels of iron associated with volcanic materials (Denton & Morrison 2009), and this could be the case in Lyttelton Harbour. A previous study carried out in Manukau Harbour sediments also showed a similar range of iron concentrations (17420 to 23940 µg g wet wt^{-1}) to that of Lyttelton Harbour, implying that iron levels are high in New Zealand marine sediments (Williamson 1992). Iron is a nutrient element and also has very low bioavailability in marine settings, so even highly elevated levels are unlikely to be toxic to marine biota. In fact, iron levels are only rarely reported in studies of marine contamination (Denton & Morrison 2009; Naidu & Morrison 1994; Redfern 2006).

4.4.1.1 SOURCES OF TRACE ELEMENTS AT THE SEA-FILL

The possible sources of cadmium and lead in the sea-fill materials could be plastics, paints, and electronic materials incorporated with the building materials. There is a sewage outfall adjacent to the T1 towards the Cashin Quay breakwater at a very close proximity to the silt curtains at the sea-fill site. This sewage outfall could also be a possible source of trace elements to the environment. In addition to cadmium and lead, significantly higher concentrations of copper, iron, and zinc were also measured in the seawater and sediments at transect line T1 relative to other sample sites. Sewage outfalls are known to be a source of trace elements into the

marine environment (Birch & Taylor 1999; Cabral-Oliveira et al. 2015; Denton & Morrison 2009; Mance 1987; Sridhara Chary et al. 2008).

The samples from transect line T1 closest to the silt curtain (1A- sample) contained a high concentrations of sediment lead (75 µg g dry wt^{-1}) in the first and second sampling rounds (68 µg g dry wt^{-1}) of this study. A previous study carried out at the proposed sea-fill site of Lyttelton Harbour before the reclamation work, showed a similar elevated level of lead (100 µg g dry wt^{-1}) (Sneddon & Barter 2009). Although a gradual decrease of lead at this sample location during the two year of sampling was noted (Figure 4.4e), the decrease was not significant. It is likely that the elevated lead concentrations represent historical contamination, possibly related to the sewage discharge and storm water runoffs (Williamson 1992). The observed gradual decrease in trace element concentrations could be due to cleaner materials from the sea-fill covering the old materials (sediment) while the sea-fill was being created.

Arsenic was another element that was elevated closer to the sea-fill site. Possible sources of arsenic in the sea-fill include treated timber, semiconductors, and glass from the demolished building materials (Denton et al. 1997). However, an alternative, more historically important source may have also been responsible for the elevated arsenic. There is a coal storage facility behind the sea-fill site. Any leaching of waste water from this source may release arsenic into the marine environment, as coal is a known source of this trace element (Nalbandian 2012), and previous monitoring work at the sea-fill site indicated the presence of coal fines in the sediments. However, previous studies have suggested that the amount of coal particles present in the sediments of were not high enough to contribute significantly to the levels of trace elements in the harbour, as New Zealand coal typically does not have high arsenic (Sneddon 2011; Sneddon & Barter 2009).

Copper and iron did not show variations with respect to distance from the sea-fill, suggesting that the sea-fill was not the only source of these elements to the surrounding seawater. However, copper and iron levels were significantly higher at the sea-fill site than at the reference site. As mentioned in Section 4.2.1 above, there are multiple sources of trace elements in LH, including a contamination site at the dry-dock in the port area, a coal storage facility adjacent to the port, outfalls of three major sewage treatment plants (one adjacent to the sea-fill), storm water runoffs from

the surrounding hills and roads, and busy shipping activities in the harbour (ECan 2008). The major source of copper and iron could be from the dry dockyard due to ship repair and maintenance work. Sources of copper to the marine environment include anti-fouling paints from ship hulls, wood preservatives and brass materials used in the ships and other port operation related activities (Denton et al. 1997), while the iron could be from the steel used for ship hulls, from the wharfs and pilling work at the port, and from other ferrous materials used in port operation activities.

Although zinc concentrations in seawater decreased significantly with distance from the sea-fill, sediment zinc concentrations did not vary with respect to distance (Figure 4.4 c, d and f), indicating that the sea-fill was not the only source of zinc. The major possible source of zinc in the harbour could be from the boating activities and ship movement at the port, as zinc is known to release from steel (Williamson 1992). Zinc can be released from the sacrificial anodes on marine vessels, paints, zinc-based alloys, brass and bronze, galvanisation work, and rubber materials (Denton et al. 1997). Other possible sources of zinc to Lyttelton Harbour could be from storm water from the surrounding hills that was contaminated with vehicle tyre rubber, sewage discharges, and roof runoff (ECan 2008; Glasby et al. 1988).

4.4.1.2 ENVIRONMENTAL PARAMETERS THAT DETERMINE TRACE ELEMENT CONCENTRATIONS IN SEDIMENTS

Sediment characteristics can determine sediment trace element concentrations. For example, sediment grain size has been recognised to affect trace element concentrations, with silt/clay fractions having higher accumulation values due to the large surface area to volume ratio of the finer particles, coupled with the strong adsorptive properties of clay materials (Glasby et al. 1988; Krumgalz et al. 1992; Luoma 1990; Williamson & Wilcock 1994). Previous investigation at the LH sea-fill site noted that the sediment texture ranged from relatively coarse gravel, sand, and shell mixtures to fine, soft muds (Sneddon & Barter 2009). This trend of sediment texture was observed visually for the sediments of this current study. Sneddon & Barter (2009) reported that the silt content increased from 6% to 64% from east (from Battery Point) to west (towards Cashin Quay breakwater), that is from T3 to T1 in this current study. This is in agreement with the higher trace element contents at T1, suggesting that sediment size may be an important factor contributing towards local

variation in trace element content. Similar findings were reported in a study of a Kiribati coastal landfill (Redfern 2006).

Numerous studies have shown that increased input rates of trace elements result in an increase in the local sediment concentrations. As such, sediments act as a sink, an effect that is most prominent in sheltered environments where the sediment movement is restricted (Denton & Morrison 2009; Denton et al. 2001; Naidu & Morrison 1994; Redfern 2006; Williamson et al. 2003). Supporting this, the concentrations of trace elements found in the transect T1 (more sheltered from the Cashin Quay breakwater) of this study were higher than other transect lines. Also, T1 was located at a position for accumulation of materials from the movement of sea currents; the formation of tidal gyres in the region turned water clockwise on ebb tide and counter-clockwise on flood tides at the breakwater (Inglis et al. 2006; LPC 2009).

It has also been recognised that metal-reactive components such as iron oxides, manganese oxides, sulphides, and organic material (e.g. humic acid, carbohydrates and proteinaceous materials) can increase in the sediment as the sediment texture becomes finer (Luoma 1990; Williamson & Wilcock 1994). Organic matter often contains negatively charged sites that can attract trace elements, thus sediments with a higher content of organic material tend to retain a higher content of trace elements (Luoma 1990; Williamson & Wilcock 1994). The organic content data of this study did not explain the variable concentrations of trace elements found with respect to the site, the distance from the sea-fill, or the sampling rounds. Copper was the only trace element significantly correlated with the organic content at the sea-fill site, whereas arsenic, cadmium, and iron were correlated with the organic content at the reference site.

Although the nature of the fill materials differs for different fill activities, the results of this study are in agreement with those of other studies suggesting that fill activities are sources of trace elements into the surrounding marine environment. This includes studies at the marine landfill site of Bermuda (Jones 2010), the coastal landfill of Suva Harbour in Fiji (Maata & Singh 2008; Naidu & Morrison 1994), the landfill of Pago Bay in Guam (Denton & Morrison 2009), and the coastal landfill of Kiribati and Te Atatu Peninsula of Auckland, New Zealand (Redfern 2006). These studies also indicated that the trace element concentrations in the sediments decrease

with distance away from the point sources.

4.4.1.3 COMPARISON TO REGULATORY GUIDELINES

Trace element concentrations in seawater at the sea-fill and the reference site were below the ANZECC trigger value (Table 4.1) for protection of 99% of species, with the exception of copper at the sea-fill site and the reference site. Copper concentrations in the seawater of the reference site in the first and the fifth sampling rounds exceeded the 99% protection levels, while almost all samples at the sea-fill site exceeded the 99% protection level in all the sampling rounds. The 95% protection levels for copper were exceeded occasionally at the sea-fill site, and one sample (at the T1) in the first sampling round exceeded the 90% protection level. The exceedance of the ANZECC trigger values for copper implies that potential adverse ecological effects are possible.

Trace element concentrations in the sediments from the sea-fill site and the reference site were generally below the ISQG-low value (Table 4.2), with the exception being lead in one sampling location closest to the sea-fill (T1-A-next to sewage outfall) at the Cashin Quay breakwater in the first and the second sampling rounds (75 and 68 µg g^{-1} respectively), which exceeded the ISQG-low value for lead (50 µg g^{-1}). The concentrations of lead at that sampling location, however, decreased to below the ISQG-low value in the subsequent sampling rounds, suggesting that the contaminated sediment may have been replaced by cleaner materials by the sea-fill, as discussed earlier. The higher value of lead in this study and a previous study (Sneddon & Barter 2009) at the sea-fill site indicates that this could have biological effects.

4.4.2 TRACE ELEMENTS IN SHELLFISH

Iron was the trace element found at the highest level in the green-lipped mussels, followed by zinc, arsenic, copper, lead, cadmium, and mercury. This trend of trace element concentration is common in green-lipped mussels in other studies where iron concentrations were reported (Kennedy 1986; Nielsen & Nathan 1975). Quantitatively, trace element concentrations of green-lipped mussels from this study were comparable to, or lower than, concentrations measured in green-lipped mussels from sites in New Zealand that were considered to be uncontaminated (Kennedy

1986; Nielsen & Nathan 1975; Whyte et al. 2009). More detailed literature comparisons of trace elements in green-lipped mussels are presented in Chapter 6 of this thesis. No sample of green-lipped mussels from the sea-fill site or the reference site exceeded the ML values for bivalve molluscs (Table 4.3). This suggests that green-lipped mussels from these locations are safe for human consumption.

With the exception of cadmium, all trace element concentrations were higher in the sea-fill site samples (BP- Battery Point adjacent to the sea-fill) than that of reference site (PG). This cadmium accumulation in the green-lipped mussels at the reference site was independent of total environmental metal burdens, which were lower in the reference site. This implies that bioavailability of cadmium at the reference site could be higher than that of the sea-fill site.

There are multiple sources of trace elements at LH, including the sea-fill site (discussed earlier), and the comparatively higher concentrations of trace elements found in the shellfish at the sea-fill site were not unexpected. The possible anthropogenic sources of trace elements at the reference site could be from storm water runoff from the adjacent hills where agricultural activities such as cattle farming take place. Although the population in the reference site area was very small compared to LH, another possible source of cadmium at the reference site was sewage outfalls from the surrounding households. Application of cadmium-containing phosphate fertilisers, sewage sludge, and manure from the farming activities are known to be the primary anthropogenic sources of cadmium to soil (Hutton 1983; McDowell et al. 2013; Thornton 1991; TRC 2005). On the other hand, one of the largest natural sources of cadmium is via volcanic activities (Hutton 1983), and both the sampling sites were located in a volcanic area, Banks Peninsula of New Zealand. Therefore, it is likely that the area may be rich in natural sources of cadmium, but this alone does not explain the higher bioaccumulation of cadmium only at the reference site. Another study of green-lipped mussels also found higher cadmium concentrations at Pigeon Bay (reference site) compared to LH (Chandurvelan 2013), and the baseline study of shellfish from the wider LH in this thesis (Chapter 6) also indicated significantly higher cadmium concentrations in the samples of the Pigeon Bay site. However, the cause of these significantly higher cadmium concentrations is unknown.

The concentrations of mercury and lead in the green-lipped mussels of the sea-fill site remained significantly higher than those of the reference site, indicating constantly higher availability of these elements at the LH site. In general, trace element concentrations in seawater and sediments were significantly higher at the sea-fill site, although mercury was not able to be analysed from either of the other matrices in this study. However, previous studies highlighted elevated levels of mercury in the vicinity of the sea-fill area (ECan 2008; Sneddon 2011; Sneddon et al. 2010). The sediment and seawater results support the higher level of lead found in the green-lipped mussels at the sea-fill site compared to the reference site.

In general, less variation in body burden was observed for the essential elements, compared to the non-essential elements. However, within the essential elements, iron concentrations were more variable than copper and zinc. The higher variability of iron in green-lipped mussels could be related to the sediment particulates taken up by the shellfish during filter feeding. These mussels were not depurated before the analysis of the whole soft tissue in this study, and it is known that shellfish can contain fine sediment particulates in the gut (Kennedy 1986), resulting in an overestimation of true body burden (Marsden et al. 2014). The higher concentrations of iron in the green-lipped mussels at the sea-fill site, compared to the reference site, could be due to the higher sedimentation in the former site, which would potentially expose the green-lipped mussels to higher levels of particulates (Kennedy 1986).

The lower variation of copper and zinc concentrations in green-lipped mussel from both sites could relate to their essentiality, and thus their regulation within a certain optimal range (Reinfelder et al. 1998; Yılmaz et al. 2010). However, these elements are known to vary in accordance with the spawning cycle, where zinc, in particular, is known to play an important role in reproduction (Banks et al. 1999; Coimbra & Carraça 1990; Miramand et al. 1991; Olsson et al. 1987). Numerous studies have shown that shellfish can accumulate trace elements in proportion to their availability in the surrounding environment and that this varies depending on season (Boening 1999; Goldberg 1986; Rainbow 1995). Although essential, at higher exposure concentrations, regulation can be overwhelmed, thus leading to bioaccumulation similar to non-essential elements (Mertz 1981; Yılmaz et al. 2010).

It is usual for non-essential trace elements such as arsenic, cadmium, mercury, and lead to be accumulated in relation to the exposure level, and these elements are stored in various organs as detoxified or metabolically available forms (Amiard et al. 2006; Berthet et al. 2003; Luoma & Rainbow 2005; Rainbow 2007). Consequently, levels of these elements are often trapped in the body for longer periods than essential elements, leading to higher body burdens.

4.5 CONCLUSIONS

The results of this study indicate that the sea-fill is a likely source of trace elements to LH, and suggest that trace elements accumulate closer to the input source. The MPI values from three different environmental matrices indicate that the sea-fill site contains higher levels of trace element contamination than the reference site (PG), implying greater anthropogenic inputs at the sea-fill site. Concentrations of copper in seawater and lead in sediments at the sea-fill site are at a level that could cause possible adverse ecological effects. Conversely, the concentrations in green-lipped mussels at the sea-fill site and the reference site mussels are safe for human consumption from the perspective of trace element exposure. The results also suggest that multiple matrix analysis is required for a complete picture of trace element content in monitoring, as the sediment and seawater results of this study indicated a higher concentration of cadmium at the sea-fill site, although the cadmium content in the green-lipped mussels indicated higher bioavailability of cadmium at the reference site. The irregular pattern of trace elements in different sampling rounds implies that concentrations can vary depending on many environmental and geochemical factors and can vary with season and the environmental matrix.

The comparisons of trace elements in sediments of sea-fill or coastal landfills presented in Table 4.2 indicate that the concentrations of trace elements in fill sites are due to a number of factors. These include the nature of the fill materials, the inputs or proximity to the source, the geology of the site, the physical transport of materials (sea current and sediment movements), and the chemical and physical properties of the sediments.

CHAPTER 5

TROPHIC TRANSFER OF TRACE ELEMENTS AT THE SEA-FILL OF LYTTELTON HARBOUR, NEW ZEALAND, AND RISK ASSESSMENT FOR CONSUMPTION OF FISH

5.1 INTRODUCTION

Continuous release of trace elements into the surrounding ecosystem can have potential long-term implications for the ecosystem (Ip et al. 2007) and seafood consumers. While consumed aquatic species can be exposed to trace elements directly from the water and sediments they inhabit (Ahlf et al. 2009; Wang & Rainbow 2008), passage through food chains is the greatest source of trace elements to aquatic animals

(Amiard-Triquet et al. 1992a; Mason et al. 2000; Mason et al. 1996). This is especially true of predatory species that inhabit the apex of aquatic food chains, and which are also then consumed by people. Seafood is a very common food source for human populations due to its nutritive properties (Andreji et al. 2006), but the consumption of contaminated seafood may expose human consumers to elevated levels of toxic elements. For this reason, regulatory limits exist that seek to minimise health impacts by setting limits for trace element concentrations in foods. It is consequently important to monitor the level of trace element accumulation and transfer across food chains in potentially contaminated areas such as near the sea-fill in Lyttelton Harbour (LH) of New Zealand (see Chapter 4 for site description).

Baseline surveys of trace elements in marine organisms such as fish and shellfish have been carried out in different harbours and coastal areas of New Zealand (Brooks & Rumsey 1974; Brooks & Rumsey 1965; Kennedy 1986; Love et al. 2003; Milne 2006; Nielsen & Nathan 1975; Peake et al. 2006; Whyte et al. 2009). Baseline data on trace elements in seawater, marine sediment, and mussels were available for LH (Inglis et al. 2006; Sneddon 2011; Sneddon et al. 2010) prior to the start of this study. In general these data characterise LH as a slightly to moderately disturbed environment. However, there are no trace element data for fish or other marine biota from LH. Furthermore, there are no data examining transfer of trace elements across coastal food chains in this setting. Given the recent presence of a sea-fill site in the harbour (Sneddon 2011), the significant recent geological disturbances of this watershed through significant earthquake activity, and the knowledge that such activity can drastically change environmental trace element levels (Hung & Ho 2014), then there is a significant need for information regarding the levels of trace elements in LH biota and its potential risk to seafood consumers.

5.1.1 SPECIES SELECTION AND FEEDING ECOLOGY

Two species of fish, spotty (*Notolabrus celidotus*) and banded wrasse (*Notolabrus fucicola*), were selected for examination. These are species which have a restricted home range (Denny & Schiel 2001; Jones 1984) and are endemic to New Zealand coastal waters (Scott 2010). This makes them suitable biomonitoring species as their accumulated trace element levels are likely to reflect the area from which they are caught (Phillips 1977; Rainbow 1995). They are easy to catch and may be

consumed by recreational fishers and other minority groups in New Zealand (Burgess 2013). Although the two species of fish in this study are not among commercially fished species, they may reflect the trace element concentrations in more commonly consumed fish species (Ministry of Primary Industry 2015). Studies from overseas have shown that accumulated trace elements in wrasse species reflect those of other fish species collected from similar habitats (Kalantzi et al. 2013).

Importantly, both these species exhibit generalist feeding habits. The spotty is an opportunistic feeder, with its diet consisting of macroalgae, copepods, amphipods (particularly in juvenile fish), bivalves, crabs, ophiuroids, hermit crabs, limpets and gastropods (important in larger fish) (Jones 1984). The banded wrasse is a generalist benthic predator species and the main components of its diet include seaweed, amphipods, isopods (primarily in juveniles), bivalves, gastropods, hermit crabs, polychaete worms and crabs (important in larger fish) (Denny & Schiel 2001; Russell 1983).

Cancer crabs (*Metacarcinus novaezelandiae*) are a relatively abundant species in harbours, estuaries and coastal areas of New Zealand (Creswell 1988; Creswell & Marsden 1990; Creswell & McLay 1990), where they are commonly found buried in the sediments or under rocks, stone and seaweeds (Bennett 1964). Cancer crabs are nocturnal, feeding on gastropods, amphipods, isopods, crabs, bivalves including cockles, small fish, sponges, coelenterates, polychaete worms and plant matter (Bennett 1964; Chatterton & Williams 1994; Creswell 1988; Creswell & Marsden 1990; Creswell & McLay 1990; Shelton et al. 1979).

Polychaete worms take up trace elements predominantly from ingestion of sediment-bound particulates, including algae, microorganisms and detritus (Fauchald & Jumars 1979). Thus, trace element burdens in polychaete worms can directly correlate with the concentrations in the sediment (Meador et al. 2004; Phillips 1990). It has been recognised that polychaete worms can act as vectors for transferring contaminants from sediments into aquatic food chains, by virtue of higher trophic levels preying on these species (Meador et al. 2004; Rainbow et al. 2006a).

Green-lipped mussels (*Perna canaliculus*) are found adhering to rocky substrates and hard materials in the intertidal zones. These bivalves are suspension

feeders that take up trace elements directly from the dissolved phase and suspended particles collected during filter feeding (Rainbow 1995). They have high filtration rates that can filter several litres of water every day (Davies & Simkiss 1996). Mussels are commonly employed as bioindicator species for monitoring of contaminants (Chan et al. 1986; Chandurvelan et al. 2015; O'Connor 2002; Salazar et al. 1995).

Green algae are primary producers, found at the bottom of most aquatic food chains. The principle mechanism of nutrient and trace element uptake by the green algae is via physicochemical adsorption to the surface (Macfie & Welbourn 2000; Robinson et al. 2006).

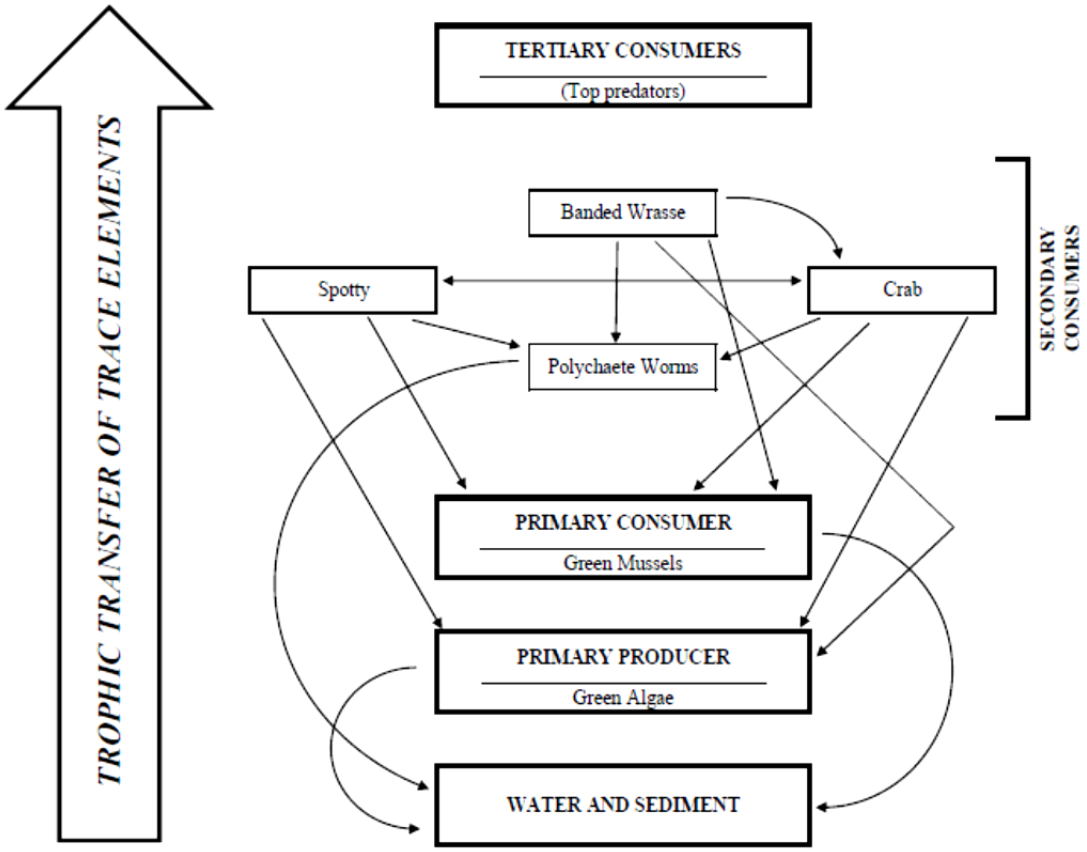


Figure 5.1: Pathways for possible trophic transfer of trace elements in the inshore coastal food chain of Lyttelton Harbour

5.1.2 STUDY OBJECTIVES

The key objectives of this study were to:

- Determine levels of trace elements in fish of Lyttelton Harbour sea-fill site
- Perform a risk assessment for consumption of these fish
- Quantify trace element concentrations in selected biota (see above) at different trophic levels of a marine food chain (Figure 5.1) around the sea-fill site at Lyttelton Harbour

5.2 MATERIALS AND METHODS

The laboratory experimental methods to extract trace elements in marine biota, and their sample preparation for chemical analysis, are provided in Chapter 2.

5.2.1 SAMPLE COLLECTION

Biota from each species (n = 12) were collected (May-June 2013) in close proximity to the sea-fill site in Lyttelton Harbour and from a reference site (Pigeon Bay- PG) (Chandurvelan et al. 2015). The species collected were green algae (*Ulva* sp.), polychaete worms, green-lipped mussels (*Perna canaliculus*), cancer crabs (*Metacarcinus novaezelandiae*), spotty (*Notolabrus celidotus*) and wrasse (*Notolabrus fucicola*) (Figure 5.2). Samples were transported to the laboratory on ice. Fish were euthanised via immersion in an overdose solution of 2 phenoxyethanol (1 g L^{-1}) as described in Chapter 3 (Sections 3.2.2.2), and crabs were chilled on a slurry of salt and ice for approximately 20 minutes, before the carapace was removed destroying the neural ganglia. All animal procedures were approved by the University of Canterbury Animal Ethics Committee.

Spotty (121-226 mm fork length, and 77-210 g whole body weight) and banded wrasse (265- 420 mm fork length, and 512-1499 g whole body weight) were collected using baited fish traps. Muscle, liver, kidney and gonad were dissected and collected in pre-cleaned and pre-weighed polyethylene centrifuge vials. Spotty gonads

were not analysed due to their small size. Crabs were collected using baited crab pots at night. The carapace lengths (84-104 mm), and widths (51-61 mm), and whole body weights (86-174 g) were recorded, and muscle from claws and legs were collected.

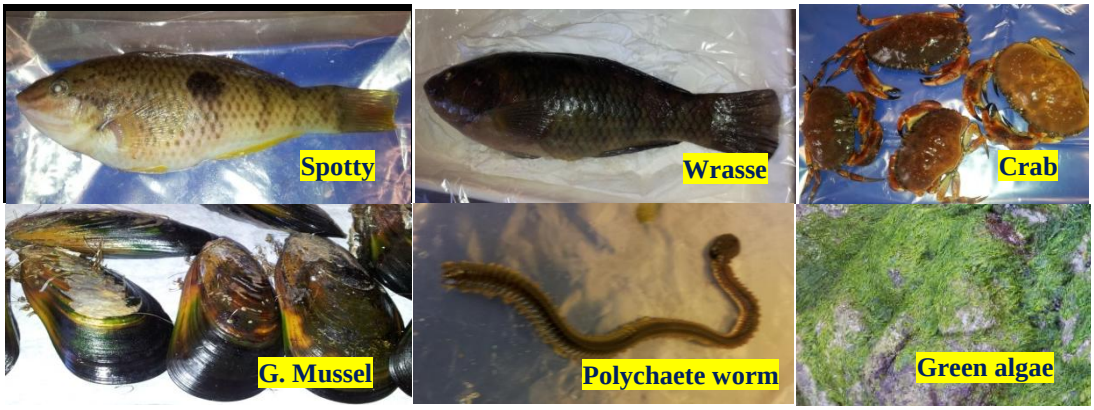


Figure 5.2: Biota collected from Lyttelton Harbour

Green-lipped mussels (86-105 mm shell length) were collected by hand from mussel beds at Battery Point and Pigeon Bay (same mussel beds as used for the quarterly sample collections for Chapter 4). The entire soft tissues of the shellfish were collected, treated and stored as described in Chapter 4 (Section 4.2.2.3). Polychaete worms were collected by hand from green-lipped mussel beds. Green algae were collected by scraping from rocks at the sea-fill site, and from wharf piles at the reference site. Wet weights of all tissue samples were recorded and the tissues were stored at -20^{o}C prior to freeze drying.

5.2.2 TROPHIC TRANSFER POTENTIAL (TTP) AND BIOMAGNIFICATION

Trace element trophic transfer potentials (TTP), representing the ability of trace elements to transfer through the food chain, were calculated using Equation 5.1 (Gray 2002; Reinfelder et al. 1998; Wang 2002), where $C_{n,f}$ is the trace element concentration (µg g wet wt^{-1}) in the consumer, and $C_{n-1,f}$ is the concentration (µg g wet wt^{-1}) in the prey.

$$TTP = C_{n,f} / C_{n-1,f} \qquad \textbf{Equation 5.1}$$

5.2.3 RISK ASSESSMENT FOR CONSUMPTION OF FISH

Trace element exposures were determined for 25+ yr adults (male – 82 kg; female – 70 kg), children of 5-6 yr (32 kg) and toddlers of 1-3 yr (13 kg) using rates of fish and other seafood consumption provided in the 2009 New Zealand Total Diet Study (NZTDS 2009). The total fish consumption rate by a male adult was 35 g per day (245 g per week); that for a female adult was 24 g per day (168 g per week); that for a child was 13 g per day (91 g per week), and for a toddler was 9 g per day (63 g per week). The risk assessments were performed for inorganic arsenic and methylmercury, as these elements do not have any known biological function in the body. Cadmium and lead, which are also considered toxic elements, were below the LOQ in the muscle of the two fish species from both LH and PG, and thus were not included in the risk assessment. Ten percent of the total arsenic was assumed to be in the inorganic form (WHO 2010b). All mercury was assumed to be methylmercury (MeHg), as it is the predominant form of mercury in fish (93-100%) (Hight & Cheng 2006; Olmedo et al. 2013). The mean, 95^{th} percentile and maximum concentrations of inorganic arsenic and mercury in the fish muscle samples were used in the trace element exposure calculations. The trace element exposure doses were calculated as described in Chapter 3 (Section 3.2.2.4).

5.2.4 STATISTICAL ANALYSIS

All statistical analyses were carried out in R© (Version 2.15.3). For trace elements, analyses were only performed for sample sets where more than 50% of the samples had values above the LOQ. In conditions where more than 50% of the samples were above the LOQ, the remaining samples below LOQ were given a value of half the LOQ. All duplicate measurements were averaged before inclusion in the statistical analysis.

All data were checked for normality by plotting probability plots. Where necessary, data were log transformed to meet assumptions of normality before analysis. Significant differences ($p < 0.05$) for trace element concentrations in biota species between the sites, among the species within a site, and differences between tissues within a fish were determined by using single factor ANOVA followed by Tukeys HSD tests.

5.3 RESULTS

5.3.1 ANALYTICAL METHOD PERFORMANCE

Standard reference material of mussel tissue (SRM-2976) and fish protein (DORM-3) were digested along with the environmental fish muscle, crab meat and shellfish samples. Bovine liver (SRM 1557c), mussel tissue (SRM-2976) and fish protein (DORM-3) were digested along with the fish organs and polychaete worm samples. Tomato leaves (SRM 1573a) were digested in duplicate along with the environmental green algae samples. The percentage recoveries of all elements in the standard reference mussel and fish protein (DORM-3) ranged from 90 to 116%, with the exception of lead in DORM-3 (36.4%- see Section 2.4.3.2). The mean percentage recoveries of all elements in certified reference bovine liver ranged from 89 to 98%, while arsenic and mercury were <LOQ. Mean percentage recoveries of cadmium, copper, iron and zinc in certified reference tomato leaves ranged from 94 to 101%, (arsenic and mercury were <LOQ), while the certified value for lead was not provided for tomato leaves (for details see Appendix C1).

5.3.2 TRACE ELEMENTS IN FOOD CHAIN SPECIES

The trace element concentrations (mean values ± SE in µg g wet wt^{-1}) in fish muscle and other soft tissues are presented in Appendix C2. The trace element concentrations (mean values ± SE in µg g wet wt^{-1}) in crab muscle, green-lipped mussels, polychaete worms and green algae are presented in Appendix C3. All results are presented in wet weights (wet wt) unless specified. Graphical comparisons of trace elements in different marine biota (for fish, the plotted value represents muscle) are presented in Figure 5.3, and the concentrations of trace elements within fish tissues are presented in Figure 5.4. For ease of comparison some of the graphs were drawn on logarithmic scale for better representation of the wide range of concentrations measured in different species.

Cadmium concentrations in crab muscle from both sites, and lead in crab muscle of PG were below the LOQ. In addition, cadmium and lead concentrations in the muscle of spotty and wrasse from both LH and PG were below the LOQ. However, cadmium and lead concentrations in fish organs (liver, kidney and gonad)

were above the LOQ. In general, the concentrations of trace elements in fish tissues were in the order: liver ≥ kidney ≥ gonad > muscle. Muscle tissue contained significantly lower concentrations than other tissues. The concentrations of trace elements in fish tissues were generally higher in the samples of LH than PG. However, cadmium and copper in liver of spotty, and liver and kidney of banded wrasse from the PG site contained significantly higher concentrations than those of LH (see Figures 5.4 b and c). For arsenic and iron, only kidney concentrations of these elements differed between the two sites, with LH showing higher concentrations than PG (see Figure 5.4 a and d). In crabs, iron and zinc were found at significantly higher concentrations in animals collected from LH than those collected from PG.

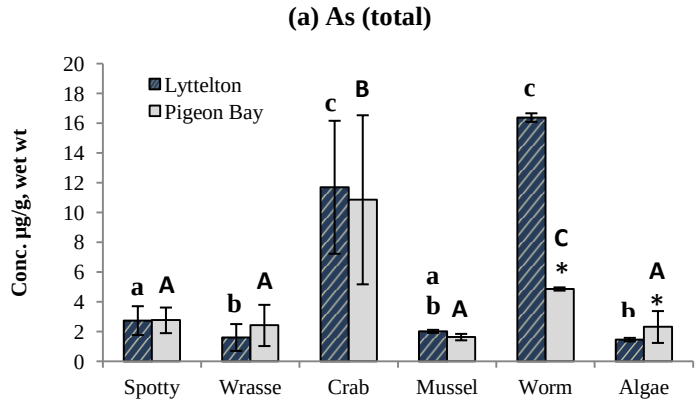
The highest mean concentrations for individual trace elements among all the species investigated were found for iron in green algae and green-lipped mussel (1577 µg g^{-1} and 148 µg g^{-1} respectively), zinc in crabs (64 µg g^{-1}), and arsenic in polychaete worms and crabs (16.4 µg g^{-1} and 11.7 µg g^{-1} respectively). In general, iron, zinc and arsenic were the most abundant elements in the investigated species in this inshore coastal food chain. Copper concentrations were at an intermediate level ranging from 0.12 to 9.62 µg g^{-1}, while cadmium (<LOQ-0.75 µg g^{-1}), mercury (<LOQ-0.28 µg g^{-1}) and lead (<LOQ-0.96 µg g^{-1}), were found at very low concentrations (Appendix C2 and C3). Interestingly, mercury was found at the highest concentrations at the top of the food chain, with the highest levels measured in the fish (banded wrasse), followed by the crabs.

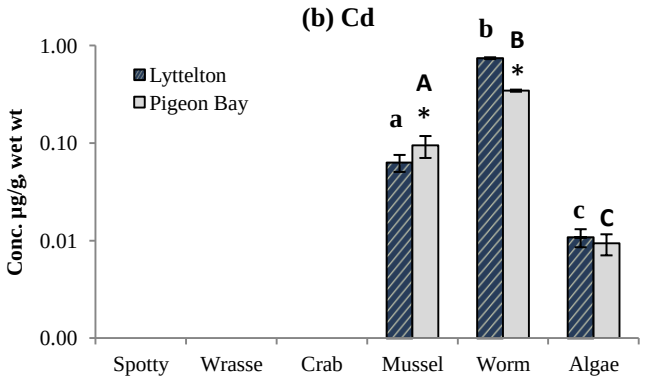
The highest mean concentration of cadmium was recorded for polychaete worms (0.75 µg g^{-1}), and the lowest in green algae (0.01 µg g^{-1}), while banded wrasse displayed the highest mean concentrations of mercury (0.28 µg g^{-1}). The highest mean copper concentrations were measured in the crabs (9.62 µg g^{-1}), and the lowest in the muscle of the spotty (0.12 µg g^{-1}). Green algae (0.96 µg g^{-1}) contained the highest mean concentrations of lead with the lowest lead levels measured in the crab (0.01 µg g^{-1}).

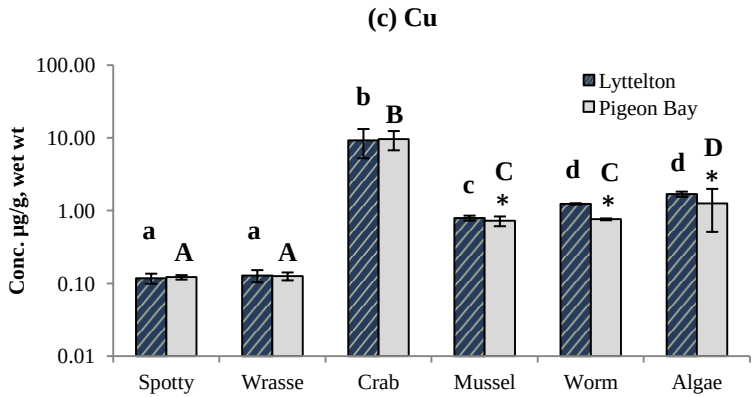
The ranking order of mean concentrations of trace elements in fish muscle tissue was zinc > arsenic > iron > copper > mercury for spotty; and the order for banded wrasse was zinc > arsenic > iron > mercury > copper. The trace element concentrations in crab muscle followed the order zinc > arsenic > copper > iron >

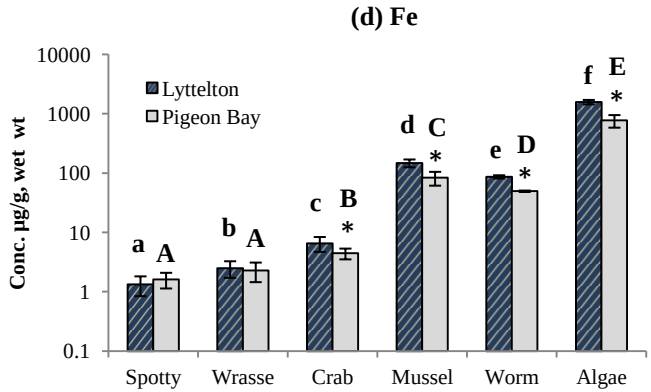
mercury > lead; while the order for the green-lipped mussels was iron > zinc > arsenic > copper > lead > cadmium > mercury. The ranking order of mean trace element concentrations in polychaete worms was iron > zinc > arsenic > copper > cadmium > lead; while for green algae the order was iron > zinc > arsenic = copper > lead > cadmium. In general, zinc, arsenic and iron presented at the highest concentrations in all the species investigated, followed by copper.

Overall arsenic, copper, mercury, lead, zinc and iron concentrations in the investigated species were higher in LH samples than those of PG (reference site). However, overall higher concentrations of cadmium were measured in the samples of the PG site.


(a) As (total)
Conc. µg/g, wet wt
Lyttelton
Pigeon Bay
Spotty
Wrasse
Crab
Mussel
Worm
Algae


(b) Cd
Conc. µg/g, wet wt
Lyttelton
Pigeon Bay
Spotty
Wrasse
Crab
Mussel
Worm
Algae


(c) Cu
Conc. µg/g, wet wt
Lyttelton
Pigeon Bay
Spotty
Wrasse
Crab
Mussel
Worm
Algae


(d) Fe
Conc. µg/g, wet wt
Lyttelton
Pigeon Bay
Spotty
Wrasse
Crab
Mussel
Worm
Algae

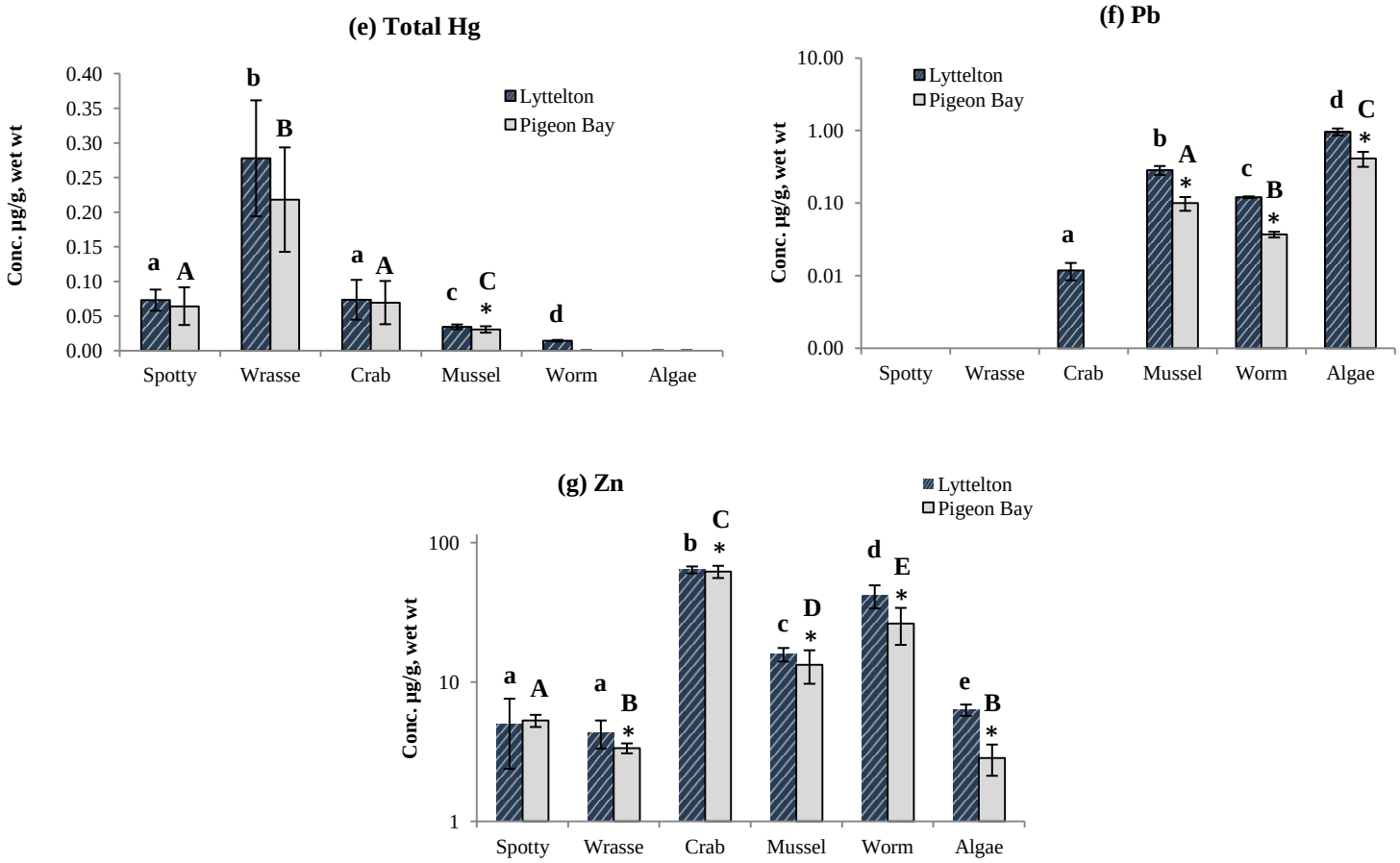
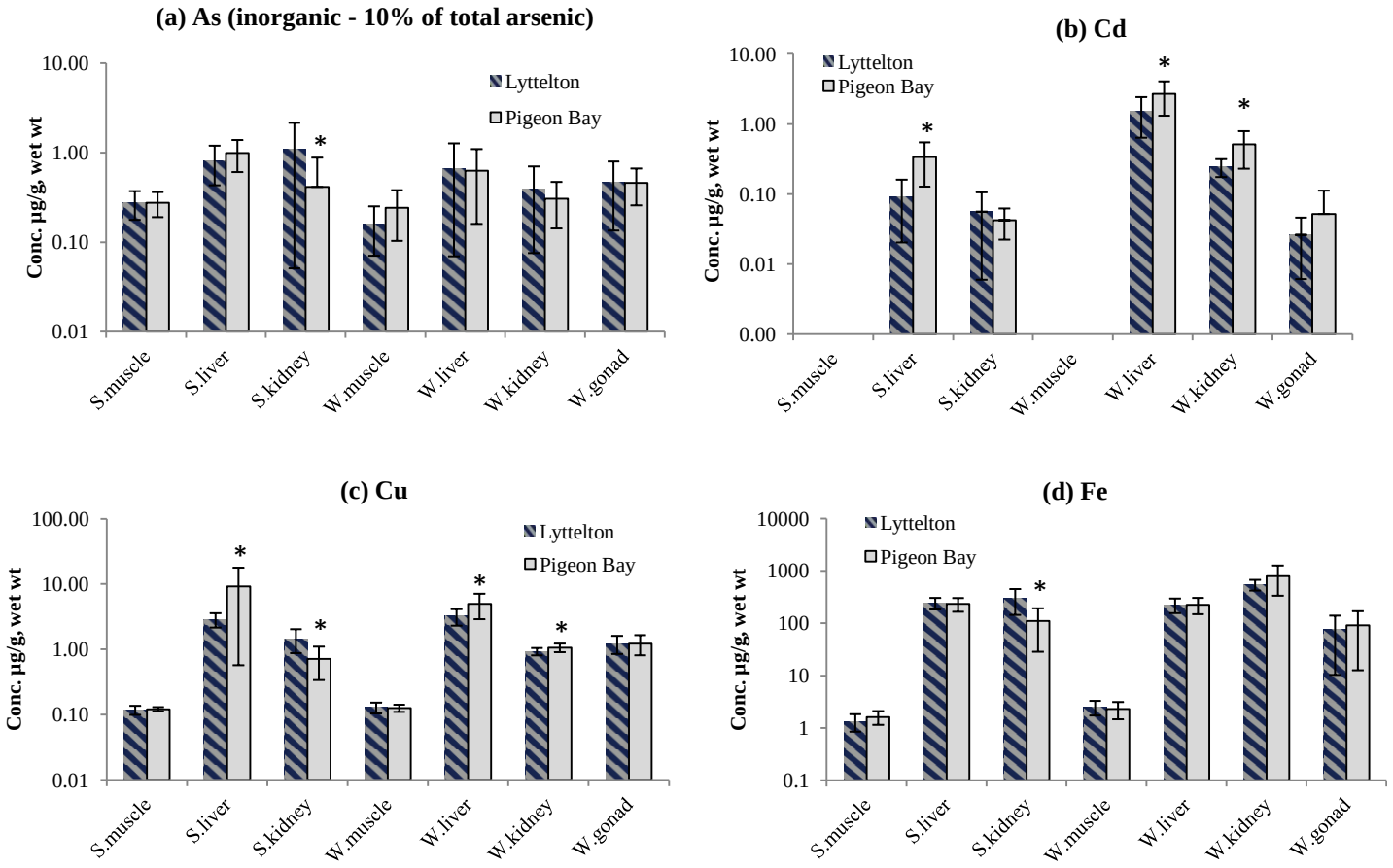


Figure 5.3: Comparison of mean trace element concentrations in biota of marine food chains. Plotted values represent means ± standard errors (n = 12). Species sharing letters are not significantly different, and asterisks indicate significant differences between sites within a species. Lowercase letters represent LH samples and uppercase letters are for PG samples. Cd was < LOQ in fish and crab muscle, Hg was < LOQ in PG worms and < LOQ for algae of both sites, Pb was < LOQ in fish muscle of both sites and in crab muscle of the PG site.


(a) As (inorganic - 10% of total arsenic)
Conc. µg/g, wet wt
10.00
1.00
0.10
0.01
Lyttelton
Pigeon Bay
S.muscle
S.liver
S.kidney
W.muscle
W.liver
W.kidney
W.gonad
(b) Cd
Conc. µg/g, wet wt
10.00
1.00
0.10
0.01
0.00
Lyttelton
Pigeon Bay
(c) Cu
Conc. µg/g, wet wt
100.00
10.00
1.00
0.10
0.01
Lyttelton
Pigeon Bay
(d) Fe
Conc. µg/g, wet wt
10000
1000
100
10
1
0.1
Lyttelton
Pigeon Bay

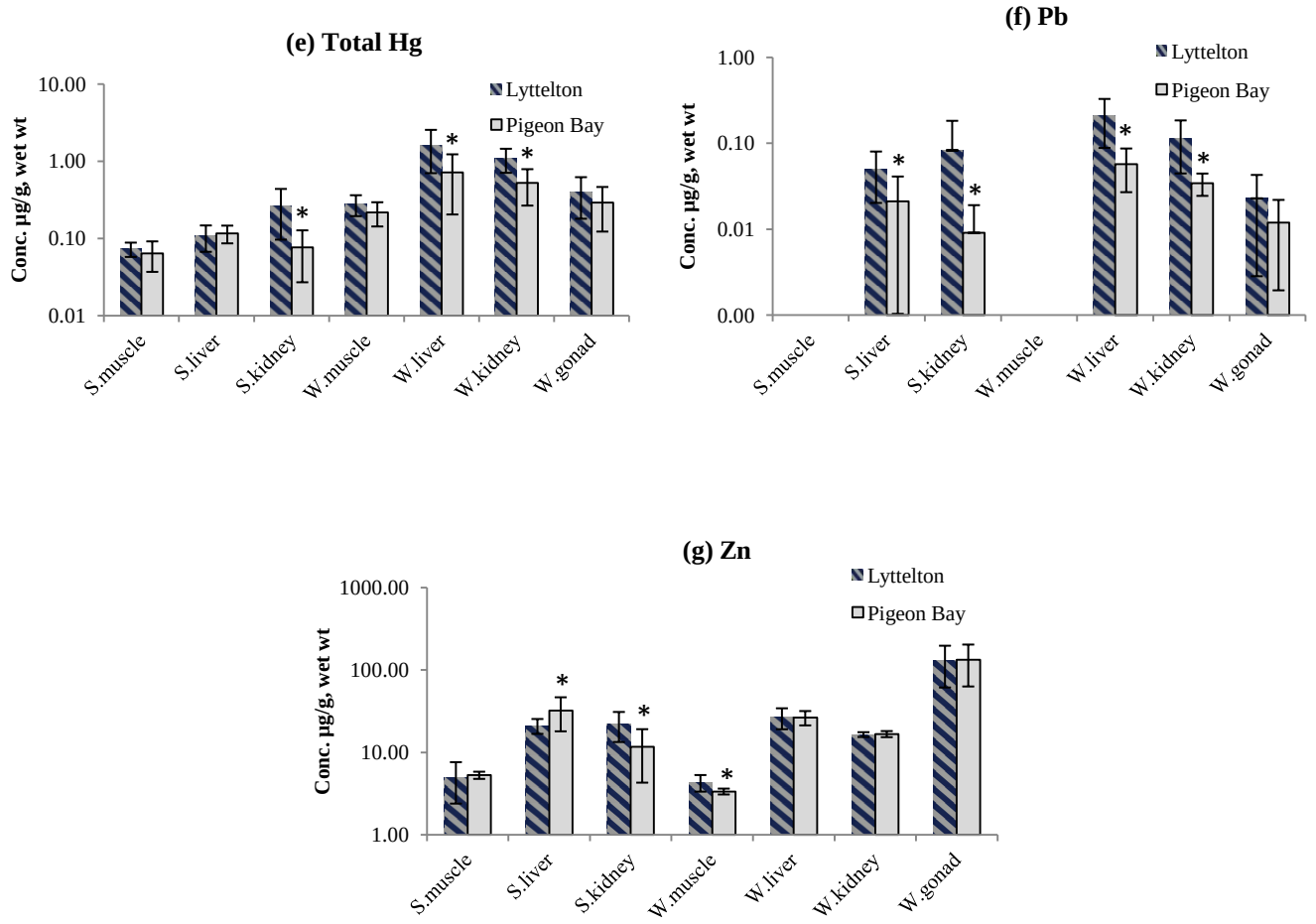


Figure 5.4: Comparison of mean trace element concentrations in different tissues of spotty (S) and banded wrasse (W) from Lyttelton Harbour (LH) and Pigeon Bay (PG). Plotted values represent means ± standard errors (n = 12). Asterisks indicate significant differences between sites within a tissue. Cd and Pb concentrations in the muscle tissues of both the fish species from both the sites were < LOQ. The inorganic arsenic was estimated as 10% of total arsenic.

5.3.3 BIOACCUMULATION AND TROPHIC TRANSFER POTENTIAL OF TRACE ELEMENTS ACROSS THE FOOD CHAIN

The ranking order of trace element concentrations between species (Table 5.1) did not reflect the trophic positions of the species in the food chain. For example, iron and lead are most concentrated in algae, even though this is at the bottom of the food chain. However, the arrangement of species with respect to concentration levels for mercury indicated a scenario more likely to reflect food chain transfer, with wrasse having the highest levels, and levels in algae below LOQ.

Table 5.1: The statistical ranking order of individual trace element concentrations between the species from highest to lowest

As	Worm, Crab	>	Spotty	>	Mussel, Wrasse, Algae					
Cd	Worm	>	Mussel	>	Algae					
Hg	Wrasse	>	Crab, Spotty	>	Mussel	>	Worm			
Pb	Algae	>	Mussel	>	Worm	>	Crab			
Cu	Crab	>	Algae, Worm	>	Mussel	>	Wrasse, Spotty			
Zn	Crab	>	Worm	>	Mussel	>	Algae, Wrasse			
Fe	Algae	>	Mussel	>	Worm	>	Crab	>	Wrasse	> Spotty

A TTP value > 1 indicates the possibility of biomagnification, whereas a TTP value < 1 indicates biodiminution or trophic dilution (Gray 2002; Reinfelder et al. 1998; Wang 2002). The calculated TTP values for the food chain transfer analysis are presented in Table 5.2. Biomagnification was observed for arsenic, cadmium, copper, mercury and zinc in the inshore food chain for LH as indicated in bold in Table 5.2. In contrast, trophic dilution was observed for lead and iron in this food chain. In general, the TTP values >1 were found in the lower food chain for cadmium, copper and zinc while TTP > 1 were observed at the higher trophic levels for arsenic and mercury.

Table 5.2: Trophic transfer potential (TTP) for individual elements between species

Species	TTP / As	TTP / Cd	TTP / Cu	TTP / Fe	TTP / Hg	TTP / Pb	TTP / Zn
Wrasse /Algae	**1.10**	0.00	0.08	0.00	0.00	0.00	0.68
Wrasse / Worm	0.10	0.00	0.10	0.03	**19.74**	0.00	0.10
Wrasse / Mussel	0.80	0.00	0.16	0.02	**8.14**	0.00	0.27
Wrasse / Crab	0.14	0.00	0.01	0.38	**3.79**	0.00	0.07
Spotty /Algae	**1.87**	0.00	0.07	0.00	0.00	0.00	0.79
Spotty/ Worm	0.17	0.00	0.10	0.02	**5.17**	0.00	0.12
Spotty / Mussel	**1.36**	0.00	0.15	0.01	**2.13**	0.00	0.32
Spotty/ Crab	0.23	0.00	0.01	0.20	0.99	0.00	0.08
Crab /Algae	**8.00**	0.00	**5.47**	0.00	0.00	0.01	**10.13**
Crab / Worm	0.71	0.00	**7.48**	0.08	**5.21**	0.10	**1.54**
Crab / Mussel	**5.80**	0.00	**11.69**	0.04	**2.15**	0.04	**4.05**
Mussel / Algae	**1.38**	**5.83**	0.47	0.09	0.00	0.30	**2.50**
Worm / Algae	**11.20**	**68.61**	0.73	0.05	0.00	0.13	**6.59**

Numbers in bold indicate values of TTP >1, indicative of bio-magnification

5.3.4 RISK ASSESSMENT FOR CONSUMPTION OF FISH

Trace element concentrations measured in the food chain species of this study were compared to the Food Standard Australia New Zealand (FSANZ) maximum allowable levels (ML) and European Commission (EC) maximum allowable values (Table 1.3, Chapter 1), which primarily assume food chain species are consumed traditionally (i.e. fish muscle is the consumed portion of the fish). However, concentrations of trace elements in organs of fish were included for the purpose of comparisons in this study as these tissues are consumed by humans in some parts of the world, including the Maldives. Concentrations of cadmium in liver and kidney of banded wrasse from both LH and PG; liver of spotty from both sites; and kidney of spotty from LH exceeded the maximum allowable value for fish (0.05 µg g wet wt $^{-1}$). Banded wrasse kidney and liver samples from both LH and PG exceeded the ML value for mercury in fish (0.5 µg g wet wt $^{-1}$). The concentrations of zinc in gonad samples of banded wrasse were more than three times higher than the ML. No sample exceeded the ML for copper. No comparison was made for iron as there was no ML value provided in either FSANZ or EC regulations. The trace element concentrations in fish muscle, fish organs, crab muscle, and green-lipped mussel did not exceed the

maximum values set by FSANZ for inorganic arsenic.

Table 5.3: Estimated weekly intake (EWI) of trace elements at different consumption rates by different age-gender cohorts at different concentrations in fish muscle tissue

		Consumption rate (g / week)	EWI of trace elements (µg / kg BW / week)			
			Inorg. As	Cd	Hg	Pb
	WHO / JECFA PTWI values		**21**	**5.6**	**1.6**	**25**
Fish consumption	**82 kg male**	245				
	Mean conc.		0.82	-	0.83	-
	95th percentile conc.		1.27	-	1.10	-
	Max conc.		1.58	-	1.13	-
	70 kg female	168				
	Mean conc.		0.66	-	0.67	-
	95th percentile conc.		1.02	-	0.88	-
	Max conc.		1.27	-	0.91	-
	32 kg child	91				
	Mean conc.		078	-	0.79	-
	95th percentile conc.		1.21	-	1.04	-
	Max conc.		1.51	-	1.08	-
	13 kg toddler	63				
	Mean conc.		1.33	-	1.35	-
	95th percentile conc.		2.06	-	**1.78**	-
	Max conc.		2.57	-	**1.84**	-

The estimated weekly intake (EWI) values for inorganic arsenic and mercury were compared to the JECFA PTWI values (Section 1.5.2). The PTWI values for inorganic arsenic and mercury were not exceeded at previously published rates of fish consumption by adults of the general population of New Zealand (Table 5.3). Hence it is unlikely to be significant risk associated with the consumption of fish harvested from LH. However, the PTWI value for mercury was exceeded for toddlers with the average fish consumption rates provided in the NZTDS at the 95th percentile and maximum concentration values measured in fish muscle.

5.4 DISCUSSION

5.4.1 TRACE ELEMENT ACCUMULATION IN THE FOOD CHAIN

The concentration of trace elements in marine food chains depends on several factors including the physicochemical properties of the habitat (e.g. water chemistry, pH, metal speciation), feeding ecology, life span and the strategies for metal handling, storage or depuration adopted by the species therein (Blackmore & Wang 2004; Otero-Romani et al. 2005; Rainbow 2002,2007). It has been reported previously that trace element concentrations can also vary with sex, age and size within a species (Fabris et al. 2006; Forsyth et al. 2004; Reinfelder et al. 1998).

5.4.1.1 FISH AND CRABS

This is the first study to examine trace element accumulation in banded wrasse (*Notolabrus fucicola*), spotty (*Notolabrus celidotus*) and the cancer crab, (*Metacarcinus novaezelandiae*). Concentrations of trace elements measured in banded wrasse, spotty and crabs were compared to other studied species with similar behaviours and ecology, where available (Tables 5.4 and 5.5).

Trace element concentrations in muscle of the two fish in this study were comparable to those previously reported for similar fish species (Table 5.4). Levels of copper in all the studied species were at levels that have been previously observed in the literature (Tables 5.4-5.7). It was notable that copper was elevated in crabs, likely a consequence of the role that copper plays in haemocyanin, the crustacean blood pigment (Depledge et al. 1993; Devescovi & Lucu 1995).

Biomagnification of cadmium, copper and zinc was noted in the current study. However, it should be noted that this could be a consequence of not depurating the samples prior to the analysis. Trace elements associated with food particles in the digestive tract may represent accumulated burdens that are not actually assimilated into the animal, thus overestimating the true trace element burden, and overestimating TTP. Depuration of biota prior to analysis can reduce this effect, but it can also lead to the loss of truly assimilated trace elements, and so could artificially lower burdens and

underestimate TTP (Marsden et al. 2014; Suedel et al. 1994). The impact of not depurating biota samples in the current study remains unknown.

Arsenic concentrations in the muscle of the two fish species investigated in this study were not significantly different between the sites. This could reflect the similar environmental arsenic levels in the two studied sites (Chapter 4). This is supported by a study of coastal species of Australia (Victoria), where the total arsenic concentrations in snapper were significantly higher in the sites of higher environmental arsenic levels (Fabris et al. 2006). In addition, it is reported that arsenic concentrations are also related to the diet, and species with similar diets can contain similar arsenic concentrations (Barwick & Maher 2003). This supports the findings of the current study as the two species of fish from this study have similar diets (Denny & Schiel 2001; Jones 1984).

The concentrations of arsenic measured in cancer crab of this study were comparable to some species of crab reported in previous studies (Table 5.5). The mean concentration of total arsenic in crabs of this study was generally higher than those of other food chain species investigated, with the exception of polychaete worms from LH sea-fill site. This observation is in agreement with previous studies on arsenic in food chains (Barwick & Maher 2003; Fabris et al. 2006; LeBlanc & Jackson 1973). LeBlanc and Jackson (1973) reported that arsenic concentrations in crab (*Cancer magister*) were the highest among fish, shellfish, and other marine invertebrates along the western coast of Canada. Fabris et al. (2006) also found relatively higher concentrations in crustaceans compared to other marine species, although the explanation for this pattern was unknown (Barwick & Maher 2003). The high content of arsenic in the crabs of this study could be attributed to the high storage capacity of arsenic (detoxified or metabolically available forms) due to a higher rate of arsenic assimilation compared to the excretion or elimination rate (Falconer et al. 1983; Phillips 1990; Wang & Rainbow 2008) in comparison to other species. Falconer et al. (1983) observed that the level of arsenic in a fish reflects the level of arsenic in the diet of the fish. Therefore, the high concentrations of arsenic in the crabs of this study also could be related to the high concentrations of total arsenic in its potential food sources, such as polychaete worms, algae and cockles. For example, the baseline survey of shellfish from Lyttelton Harbour showed higher total arsenic in

cockles compared to other bivalves, Chapter 6 (Section 6.3.2.1). Although the green algae in this study contained low total arsenic concentrations, the diet of crab may contain other species of algae. It is reported, for example, that brown algae, a species available in the harbour, accumulates higher amounts of arsenic than green algae (Maher & Clarke 1984; Sanders 1979).

Banded wrasse contained levels of mercury higher than those of spotty, but the levels of both fish were comparable to those in other near-coastal fish species (see Table 5.4). Higher mercury levels in banded wrasse might relate to a slightly different feeding niche. Although their diets have previously been reported as similar, there is greater divergence in prey items at adult life-stages (Denny & Schiel 2001; Jones 1984), possibly placing the banded wrasse at a higher trophic level than the spotty.

Numerous studies indicate higher concentrations of mercury are found in large predatory fish species, and in general the mercury concentrations increase with age, size and trophic level (Bowles et al. 2001; Fabris et al. 2006; Monteiro & Lopes 1990; Storelli & Marcotrigiano 2000; Storelli et al. 2002; Zhu et al. 2012). The higher mercury level in banded wrasse could therefore relate to its comparatively larger size (512-1499 g) relative to spotty (77-210 g) (Forsyth et al. 2004; Storelli & Marcotrigiano 2000). Size could also explain why both these species in general have lower mercury levels than larger, longer-lived species such as tuna and swordfish (Love et al. 2003; Olmedo et al. 2013; Storelli et al. 2012; Zhu et al. 2012). Due to the high concentrations of mercury found in some fish species, food regulatory authorities such as Health Canada recommend limiting the number of meals of large predatory fish such as shark, swordfish and tuna, to one per week (Forsyth et al. 2004).

No significant differences in mercury concentrations were found in the crabs from the two sampling sites. The total mercury concentrations measured for cancer crabs of this study were comparable (Table 5.5) to the blue swimmer crabs (*Portunus pelagicus*) from Northeastern Mediterranean coastal waters (Balkas et al. 1982), but were comparatively lower than the total mercury in the claw meat of king crabs (*Pseudocarcinus gigas*) from the coast of western Victoria (Australia) (Turoczy et al. 2001). As described above for fish, this likely reflects relative size, age and placement in the trophic cascade. The low concentrations of mercury in the potential food items of the crab in this study (shellfish, polychaete worms and algae), would also play a

role.

The lead concentrations in the LH crabs were measurable while the lead in crabs of PG site were <LOQ (Appendix C3), indicating that environmental levels play an important role in the bioaccumulation of lead in this species (Sections 4.3.2 and 4.3.3). Lead concentrations in the cancer crabs of this study were lower than the lead concentrations measured in blue crabs (*Portunus pelagicus*) from Northeastern Mediterranean coastal waters (Balkas et al. 1982), and blue crabs (*Callinectes sapidus*) from three different sites of Iskenderun Bay of Turkey (Türkmen et al. 2006) (see Table 5.5). Blue crabs showed variations associated with site contamination, but even the concentrations of lead in the blue crabs from the cleanest site in Iskenderun Bay contained comparatively higher concentrations of lead than those of the cancer crabs in this study. The lower concentration of lead in the crabs of this study could be linked to lower bioavailability of lead from LH compared to Iskenderun Bay. The non-essentiality of lead means that it is less likely to be regulated to an optimal level in an organism, and instead may accumulate in accordance with the exposure levels (Blackmore & Wang 2004; Rainbow 1985; Roesijadi 1992; Wang 2002; Wang & Fisher 1998). This theory is consistent with the outcomes of the current study.

The concentrations of copper, iron and zinc measured in muscle of spotty and banded wrasse in this study were comparable to those previously reported for other fish species (gurnard, snapper and tarakihi) of New Zealand (Brooks & Rumsey 1974) (Table 5.4). Concentrations of these elements are subject to homeostatic regulation up to certain threshold values as they are essential to these organisms (Reinfelder et al. 1998; Wang & Rainbow 2008). The levels of essential elements are generally regulated within a species by adjusting the rate of assimilation and excretion (Luoma & Rainbow 2005; Rainbow 1993,2007; Reinfelder et al. 1998). However, at higher exposure concentrations, regulation can be overwhelmed, thus leading to bioaccumulation. For example, zinc is essential, yet the concentrations of zinc in snapper (*Pagrus auratus*) from the coast of Victoria (Australia) differed as a function of environmental levels (Fabris et al. 2006).

The copper concentrations in crabs from both the sampling sites in this study were not significantly different, and the concentrations in crabs of this study did not reflect the concentrations of copper in potential food sources, such as in shellfish and

polychaete worms. The copper concentrations in crabs were over 5-fold higher than in the green algae at the bottom of the food chain, the next biggest accumulator of copper. Several studies also report higher concentrations of copper in crabs (Balkas et al. 1982; Barwick & Maher 2003; Fabris et al. 2006; Turoczy et al. 2001). This suggests that the high accumulation of copper is required for certain physiological processes. This is likely to be attributed to the haemolymph pigment haemocyanin, a copper containing blood pigment in crustaceans (Depledge et al. 1993; Devescovi & Lucu 1995).

Significantly different levels of iron were found between crabs from the two sampling sites of the current study. The higher concentrations of iron in LH crabs likely reflect the significantly higher concentrations of iron measured in the crab diet organisms (shellfish, green algae, polychaete worms) from LH compared to PG. Additionally, iron concentrations were significantly higher in seawater and sediments of LH than PG (see Sections 4.3.2 and 4.3.3). This finding is supported by a study of blue crabs from Iskenderun Bay, where higher concentrations of iron were found in sites of higher iron contamination (Türkmen et al. 2006).

The zinc concentrations in the muscle of crab captured from the two sites were not significantly different. However, the zinc concentration in the claw muscle of the relatively large king crab (*Pseudocarcinus gigas*) from Australia (Turoczy et al. 2001) were two-fold higher than that of the cancer crab of this study. The high zinc concentration in king crab could be related to its large size, as Turoczy et al. (2001) found positive correlations between carapace size and zinc concentration. Although zinc is an essential element, changes in tissue concentrations with respect to crab size imply a changing physiology with mass (Turoczy et al. 2001), the reasons for which are unknown. However, this could be due to reproduction related effects. As zinc is important in the reproduction cycle, and it would expected that larger crabs are more fecund, they may have a higher demand for zinc, and thus a higher tissue burden.

Zinc, iron and copper are essential elements and play important roles in growth, cell metabolism and survival of most animals including crustaceans. Hence, the relatively high levels of these elements can be attributed to their essentiality to these organisms (Pourang et al. 2004). Essential trace elements are likely to be regulated by the organisms, and thus will remain in a certain range unless the

homeostatic mechanism is overwhelmed by exposure levels, leading to accumulation (Rainbow 1985; Rainbow 2007).

The low concentrations of cadmium and lead in the muscle of fish species could be due to low dietary assimilation of these non-essential elements (Long & Wang 2005; Wang & Fisher 1999). Correspondingly these elements do not bio-magnify when transferred from food/prey items at the bottom of the food chains to fish (Wang 2002; Wang & Rainbow 2008). However, it should be noted that these non-essential elements can still biomagnify if environmental levels are sufficiently high, as they are not subject to regulation in the body (Suedel et al. 1994).

The concentrations of cadmium and lead in the soft tissues of fish followed similar patterns (liver ≥ kidney ≥ gonad > muscle) to other fish species reported in previous studies (Andres et al. 2000; Brooks & Rumsey 1974; Canli & Atli 2003; Papagiannis et al. 2004), although the concentrations were not always comparable quantitatively. Similar to the non-essential elements, essential element concentrations in the soft organs of fish in this current study also generally followed similar pattern. The exception to this was that at times zinc in gonads was higher than in kidney and liver, a phenomenon also observed in the study of Brooks & Rumsey (1974) and Thilafushi Island of Maldives (Appendix A6) for some fish species. This could be due to the variation in metabolic status, which will be influenced by spawning periods and seasonal changes, and will especially impact levels of zinc owing to its important roles in reproduction (Olsson et al. 1987). Sex and species-specific differences in metabolic requirements may also be important (Wang & Rainbow 2008).

Table 5.4: Comparisons of trace element concentrations in fish (µg g wet wt $^{-1}$) with other studies

Species	Total As	Cu	Fe	Hg	Zn	Ref
Wrasse (*Notolabrus fucicola*)	0.52 - 5.14	0.10 - 0.16	1.47 - 4.27	0.16 - 0.38	3.28 - 5.27	This study
Spotty (*Notolabrus celidotus*)	1.23 - 5.30	0.10 - 0.19	1.03 - 2.52	0.03 - 0.15	3.69 - 5.94	This study
Gurnard (*Trigla kumu*)		0.34	4.2		5.5	1
Snapper (*Chrysophys auratus*)		0.11	2.5		3.7	1
Tarakihi (*Chilodactylis macropterus*)		0.28	3.4		2.8	1
Rockfish (*Sebastes* sp.*)*	<0.3 - 2.6					2
Gold striped sardine (*Sardinella gibbosa*)	2.37					3
Yellow fin bream (*Acanthopagrus australis*)	0.1 - 2.4	0.1 - 2.0		0.03 - 0.81	4.85	4
Sea mullet (*Mugil cephalus*)	0.1 - 3.8	0.2 - 2.8		0.03	4.24	4
Snapper (*Chrysophrys auratus*)	0.4 - 4.4	0.2 - 1.5		0.06 -1.94	5.30	4
Snapper (*Pagrus auratus*)	2.5 - 12.1	0.2 - 0.3		0.09 - 0.20	3.1 - 7.5	5
Groper (*Polyprion oxygenios*)				0.25		6
Gurnard (*Chelidonichthys kumu*)				0.24		6
Snapper (*Chrysophrys auratus*)				0.2		6
Blue cod (*Parapercis colias*)				0.07		6
Red cod (*Pseudophycis kachus*)				0.09		6
Blue warehou (*Seriolella brama*)				0.08		6
Tarakihi (*Nemadactylus marropterus*)				0.09		6
Anchovy (*Engraulis encrasicholus*)	0.19			0.02		7
Sardine (*Sardina pilchardus*)	0.56			0.03		7
Yellow gurnard (*Trigla lucerna*)				0.01 - 0.53		8
Anchovy (*Engraulis encrasicholus*)				0.02 - 0.21		8
Sea bream (*Pegellus erythrinus*)				0.05 - 0.70		8

LOQ-Limit of quantification; Ref: (1)- (Brooks & Rumsey 1974), (2)- (LeBlanc & Jackson 1973), (3)- (Ruangwises & Ruangwises 2011), (4)- (Bebbington et al. 1977), (5)- (Fabris et al. 2006), (6)- (Love et al. 2003), (7)- (Olmedo et al. 2013), (8)- (Storelli 2008)

The concentrations of all trace elements measured in fish organs (liver, kidney and gonad) were generally significantly higher than those of muscle tissue in both the fish species investigated. It has been reported that trace elements preferentially bioaccumulate at higher levels in liver, kidney, gonad and gills, as these tissues are metabolically active (Allen 1995; Andres et al. 2000; Canli & Atli 2003; Moiseenko & Kudryavtseva 2001; Romeo et al. 1999; Ünlü et al. 1996; Yilmaz 2003). The liver is considered the main storage and detoxifying organ, gonad is a reproductive tissue, while kidney is an important excretory organ in fish (Amiard et al. 2006; Olsson et al. 1987; Wang & Rainbow 2008,2010). The significant variation of trace element concentrations in different soft tissues of the two fish species in this study could be explained as a result of the capacities of these soft organs to induce metal-binding proteins such as metallothioneins (Amiard et al. 2006; Engel & Brouwer 1984; Marie et al. 2006; Rainbow 2002; Wang & Rainbow 2010). These sulfur-containing proteins have been characterised as the primary molecules for detoxifying trace elements (Rainbow 2002; Rainbow et al. 2006a; Roesijadi 1981; Wang & Rainbow 2010). Structures of such metal detoxifying proteins consist of a variety of functional groups (primarily the thiol residues of cysteine amino acids) that have high affinity for trace elements, and are capable of reducing the bioavailability of trace elements as part of a detoxification process (Pourang et al. 2004; Roesijadi 1992; Wang & Rainbow 2010).

The lower, and yet relatively constant, levels of trace elements in the muscle tissue of fish is accounted for by mass dilution. The muscle tissue contributes the highest mass of the whole body relative to other organs (liver and kidney), and thus the distribution of accumulated trace element into a bigger mass will make the muscle tissue less concentrated compared to the smaller organs (Meador et al. 2004; Phillips 1977; Reinfelder et al. 1998). Similar concentrations of trace elements can be found in muscle tissues of fish regardless of environmental contamination level, but the concentrations in soft organs such as liver and kidneys can often reflect the contamination levels in the surrounding environment (Moiseenko & Kudryavtseva 2001; Phillips 1977).

Table 5.5: Comparisons of trace element concentrations in crab in µg g wet wt $^{-1}$ (µg g dry wt $^{-1}$) with other studies

Species	Total As	Cu	Fe	Hg	Pb	Zn	Ref
Cancer crab (*Metacarcinus novaezelandiae*)	7.3 - 16.3 (35.5- 79.2)	8.6 - 13.4 (41.6 - 64.6)	4.4 - 7.6 (21.1 - 36.8)	0.04 - 0.13 (0.20 - 0.64)	0.01 - 0.02 (0.06 - 0.10)	55.0 - 69.6 (267 - 336)	This study
Crab (*Cancer pagurus*)	16.8						1
Crab (*Cancer pagurus*)	8.0 - 38.2						2
Crab (*Cancer magister*)	2.2 - 37.8						3
King crab (*Pseudocarcinus gigas*)		(60)		(1.2)		(650)	4
Blue crab (*Callinectes sapidus*)		(36.6)	(7.25 - 23.27)		(2.67- 4.30)		5
Blue crab (*Portunus pelagicus*)		1.7 - 21.7	1.4 - 6.3	0.03 - 0.30	0.27	19.5 - 47.9	6
Benthic crab (*Dorippe granulata*)			(171.6)				7

LOQ-Limit of quantification; Ref: (1)- (Sirot et al. 2009), (2)- (Falconer et al. 1983), (3)- (LeBlanc & Jackson 1973), (4)- (Turoczy et al. 2001), (5)- (Türkmen et al. 2006), (6)- (Balkas et al. 1982), (7)- (Depledge et al. 1993).

Table 5.6: Comparisons of trace element concentrations in green algae in µg g wet wt $^{-1}$ (µg g dry wt $^{-1}$) with other studies

Species	Total As	Cd	Cu	Fe	Pb	Zn	Ref
Chlorophyceae (*Ulva* sp.)	1.46 - 2.31 (8.3 - 17.4)	0.01 (0.05 - 0.06)	1.26 - 1.69 (9.7 - 10.8)	768 -1577 (9007 - 3919)	0.41 - 0.96 (6.3 - 29.8)	2.84 - 6.31 (14.5 - 36.0)	This study
Chlorophyceae	0.4 - 3.9						1
Chlorophyceae	(6.3 - 16.3)						2
Chlorophyceae	(10.7)						3
Chlorophyceae (*Ulva rigida)*		(0.1 - 2.5)	(1.1 - 4.3)	(84.7 - 119.3)	(2.1 - 5.5)	(39.0 - 82.5)	4

Ref: (1)- (Sanders 1979), (2)- (Maher & Clarke 1984), (3)- (Tukai et al. 2002), (4)- (Haritonidis & Malea 1999).

Table 5.7: Comparisons of trace element concentrations in green-lipped mussel and polychaete worms in µg g wet wt $^{-1}$ (µg g dry wt $^{-1}$) with other studies

Species	Total As	Cd	Cu	Fe	Hg	Pb	Zn	Ref
Green-lipped mussel (*Perna canaliculus*)	1.21 - 2.18 (8.7 - 12.0)	0.05 - 0.14 (0.26 - 0.73)	0.61 - 0.87 (3.3 - 4.8)	58 - 180 (310 - 972)	0.02 - 0.04 (0.10 - 0.22)	0.08 - 0.33 (0.44 - 1.9)	8.6 - 18.6 (46.1 - 100)	This study
(*Perna canaliculus*)	1.56 - 2.97	0.07 - 0.75			0.03	0.03 - 0.10		1
(*Perna canaliculus*)	(10.50)		(8.6)	(411)		(14.1)	(66.8)	2
(*Perna canaliculus*)		0.10 - 1.00	0.2 - 28.0	26 - 280	0.04 - 0.19	0.1 - 7.8	0.5 - 28.0	3
Blue mussel								
(Mytilus galloprovincialis)			0.55 - 1.34		0.006 - 0.012	0.22 - 1.48	28.0 - 49.8	4
(Mytilus edulis aoteanus)	1.10							5
Polychaete worm	4.7 - 16.7 (21.6 - 77.3)	0.35 - 0.75 (1.6 - 3.6)	0.74 - 1.29 (3.4 - 5.9)	46.9 - 101 (221 - 465)	<LOQ - 0.02 (<LOQ - 0.08)	0.03 - 0.13 (0.17 - 0.59)	18.5 - 56.2 (120 - 269)	This study
Polychaete worm	(32.1 - 107)							6
Polychaete worm	(2.0 - 14.8)							7
Polychaete worm	(>1000)							8
Polychaete worm	(>2000)							9
Polychaete worm	(8.8 - 117)	(<0.2 - 0.6)	(9.4 - 858)	(427 - 1521)		(2.1 - 34.5)	(69 - 201)	10

LOQ-Limit of quantification; Ref (1)- (Whyte et al. 2009), (2)- (Kennedy 1986), (3)- (Nielsen & Nathan 1975), (4)- (Milne 2006), (5)- (Robinson et al. 1995)(6)- (Dean et al. 1986), (7)- (Watts et al. 2013), (8)- (Meador et al. 2004), (9)- (Gibbs et al. 1983), (10)- (Rainbow et al. 2006a).

5.4.1.2 POLYCHAETE WORMS AND GREEN-LIPPED MUSSELS

Marine invertebrates, such as polychaete worms and bivalve molluscs have acquired remarkable and diverse strategies for accumulating trace elements (Berthet et al. 2003; Rainbow et al. 2006b). Trace element concentrations in bivalves and polychaete worms are controlled by a variety of physiological and biochemical responses including induction of metallothionein for detoxification, rate of excretion and assimilation efficiency, chemical forms of detoxified trace elements, trace element geochemistry and different elements being handled differently by different species (Reinfelder et al. 1998; Wang & Rainbow 2008). For example, these invertebrates can compartmentalise and store trace elements that have accumulated into different components such as metal-rich granules (MRG), cellular debris, organelles, metallothionein-like proteins, and other (heat-sensitive) proteins. Some of these detoxified forms such as the metallothionein-bound trace element fractions are often available for trophic transfer upon ingestion by predator, whereas the metal rich granules are less available for efficient assimilation by the predator, hence, subject to elimination (Rainbow et al. 2006b; Rainbow & Smith 2010; Wallace et al. 2003; Wallace & Luoma 2003).

Trace element concentrations in polychaete worms and green-lipped mussels were compared to previous studies (Table 5.7), and found to differ from previously reported values. Some authors describe variation of trace element concentrations in polychaete worms in terms of feeding guild, habitat type, exposure level and species-specific physiology (Gibbs et al. 1983; Meador et al. 2004; Wang & Rainbow 2008; Waring & Maher 2005). Of these it is thought that the species-specific physiology of the polychaete worms is the most important factor explaining differences in accumulation (Gibbs et al. 1983; Rainbow et al. 2004; Waring & Maher 2005). For example, a littoral polychaete worm *Nereis diversicolor* from an estuary of England was found tolerant to extreme levels of copper, and accumulated abnormally high body concentrations, whereas another species of polychaete worm from the same site did not show elevated levels (Rainbow et al. 2004).

The total arsenic concentrations in polychaete worms of this study were comparable to polychaete worms from the Gulf of Nicoya, Costa Rica, a relatively clean environment with only limited anthropogenic sources (Dean et al. 1986). Lower

arsenic concentrations than this study were observed for polychaete worms from the Sundarban mangrove, India (Watts et al. 2013), and the differences in the concentrations between species within that study were explained in terms of species-specific differences in arsenic metabolism and detoxification. The very high accumulation of arsenic reported by Gibbs et al. (1983) in polychaete worms (*Tharyx marioni*) from England (2000 µg g^{-1}, dry wt), was also explained in terms of species-specific differences. This arsenic hyperaccumulator displayed levels significantly greater than other species of polychaete worms from the same site. Similarly, Meador et al. (2004) also found that some species of polychaete worms (e.g. Family Lumbrineridae) in Alaska and California contained significantly higher concentrations of arsenic (>1000 µg g^{-1}, dry wt) than other species from the same site. However, Meador at al. (2004) also found strong correlations between arsenic concentrations in the polychaete worms and the associated sediments, suggesting physiology alone may not explain differences in accumulation.

Although polychaete worms of LH contained significantly higher concentrations of arsenic than PG, the concentrations of sediment arsenic between the sites were not different (Section 4.3.3). Similarly, Waring & Maher (2005), while finding no direct correlation between sediment and polychaete worm arsenic concentrations, reported that detrital organic material often contains arsenic-rich macroalgae, a potential food source for the polychaetes. Consequently, the higher arsenic concentrations found in the polychaete worms of LH relative to polychaete worms of PG could be related to the ingestion of a more arsenic-rich algal food source. This explanation could also apply to the significantly higher concentrations of arsenic in the green-lipped mussels of LH compared to PG, as shellfish are suspension feeders that can obtain algae from filtering water.

The wide range of copper concentrations in polychaete worms reported in the literature between different sites (Table 5.7) could be explained in terms of the metabolically available fractions of copper at those sites, and the capacity of certain species to accumulate and/or regulate trace elements (Berthet et al. 2003; Rainbow 2002; Rainbow et al. 2006a). In this regard, higher bioaccumulation of copper from sites with low total copper levels, and vice versa, has been reported due to variation in the bioavailable copper fraction (Berthet et al. 2003; Rainbow et al. 2006a). This

indicates that bioavailability, and not total copper, is the main driver of copper accumulation, at least in polychaete worms (Berthet et al. 2003; Rainbow et al. 2006a).

Zinc concentrations in the polychaete worms of this study were generally comparable to those of other species (Berthet et al. 2003). This may relate to the ability of some marine species to regulate their zinc body burden to a relatively constant level (Berthet et al. 2003). Similarly, the generally comparable results obtained for copper and iron may also relate to their essentiality, and the ability of some marine organisms to regulate their body burdens of these elements to relatively constant levels by regulatory mechanisms such as excreting any excess (Rainbow et al. 2006a). It has been reported that zinc is better regulated than copper in most species even at higher contaminant exposures (Berthet et al. 2003; Rainbow 2002; Rainbow et al. 2006a).

Polychaete worms often contain large amounts of ingested sediment in their gut, and thus without depuration, bioaccumulation values may include trace elements that are ingested but not absorbed (Flegal & John 1977; Waring & Maher 2005). The polychaete worms in this study were analysed without depuration to reflect the actual amount of trace elements that would be ingested by the predators of these invertebrates. However, the bioavailability of this ingested sediment is likely to be lower than that of biologically-assimilated trace elements, and thus may distort the relationships between levels of trace elements across trophic levels (Rainbow 2002; Rainbow et al. 2004; Rainbow et al. 2006a,b; Rainbow & Smith 2010). Similarly, trace elements taken up by biota may also differ in their bioavailability. Marine invertebrates often detoxify and store trace elements in metabolically unavailable forms such as metal rich granules (Luoma & Rainbow 2005; Nott & Nicolaidou 1990; Rainbow et al. 2006a,b). Thus the relative level of biologically active trace element is key to determining trace element transfer in food webs (Rainbow & Smith 2010).

Cadmium and lead concentrations in polychaete worms from the LH sea-fill site were higher than those of PG, reflecting environmental levels (Section 4.3.3). The cadmium concentrations measured in the polychaete worms of this study were higher than those previously reported for polychaete worms from potentially contaminated estuaries in Southwest England (Rainbow et al. 2006a), while lead

concentrations in the polychaete worms of this study were lower than the polychaete worms of the same English study (Table 5.7). This could be due to the relative levels of these trace elements and their mechanisms of uptake and detoxification. For example, the polychaetes of this study may have more capacity to handle or store cadmium, and in so doing pathways for the uptake and storage of lead may be compromised, owing to shared components of the detoxification process for these two elements (Rainbow et al. 2006a).

Comparisons of trace element levels in the green-lipped mussels of this study were made with other available literature (Table 5.7). More detailed comparisons of trace elements in bivalve shellfish species are discussed in Section 6.4. With the exception of cadmium, all other trace elements investigated in green-lipped mussels contained significantly higher concentrations in the samples of LH than PG. In general, the higher concentrations of trace elements in the green-lipped mussels of LH could be due to the higher contamination levels measured in LH seawater and sediments compared to the PG site (Sections 4.3.2 and 4.3.3). Although cadmium concentrations in the mussels were higher for PG, the seawater and marine sediments of LH site contained significantly higher concentrations of cadmium than the PG site. Discussion on the higher contents of cadmium found in green-lipped mussels of PG was presented in Section 4.4.2.

5.4.1.3 GREEN ALGAE

With the exception of arsenic, all measureable trace elements in the green algae of this study were significantly higher in concentration in the samples from LH compared to PG. The green algae from PG were collected from wharf piles, while at LH they were collected from submerged rocks. One possible reason for the higher arsenic level in PG algae is therefore due to an exposure to higher arsenic concentrations. Treated wood, such as that used for wharf pilings, is known to contribute arsenic to the environment (Akter et al. 2005) and it is therefore likely that the wharf could have contributed towards an elevated arsenic exposure to the green algae collected from PG.

Cadmium, lead, and zinc concentrations in green algae of this study were comparatively lower than concentrations in the green algae (*Ulva rigida*) from

Thermaikos, Gulf of Greece (Haritonidis & Malea 1999). In contrast, concentrations of copper and iron in the green algae of this study were higher than the concentrations of these elements in the green algae from the Greek study. The uptake of trace elements by green algae depends on three main factors; the environmental availability, metabolic state of the algae, and algal species (Maher & Clarke 1984; Phillips 1990; Sanders 1979; Tukai et al. 2002), and some researchers argue that the most important of these factors is the species of algae (Phillips 1990; Sanders 1979).

Overall, arsenic, copper, mercury, lead, zinc and iron concentrations were higher in LH samples than samples sourced from PG (the reference site). The likely reasons for the higher concentrations of trace elements in LH samples (with the notable exception of cadmium in green-lipped mussels and arsenic in algae) could be higher trace element availability at the LH (sea-fill) site. This may be explained by the multiple sources of trace element input to the harbour, including the sea-fill, the dry-dock yard, storm water runoff, sewage outfalls from the adjacent towns, port operations activities, and the coal stock yard facilities (Chapter 4). The possible explanations for the higher concentrations of cadmium in the green-lipped mussels of PG site were discussed in Section 4.4.2.

The general principle observed across the different marine organisms was that essential elements are regulated within a certain optimal range, while non-essential trace elements are not subject to regulation. Accumulated trace elements that are beyond the organisms requirements are detoxified and stored, either in the form of less bioavailable metal rich granules or more bioavailable metallothionein (Rainbow & Smith 2010). Although the bioavailability of these two fractions differs, both lead to accumulation and elevated trace element body burdens (Rainbow 2002; Rainbow et al. 2006b; Wallace et al. 2003; Wallace & Luoma 2003). In general, the levels of trace elements measured in different organisms of this study reflected environmental levels, with higher trace element levels in the organism being found at sites of higher trace element contamination. This has also been observed in previous studies (Berthet et al. 2003; Chandurvelan et al. 2015; Kennedy 1986; Luoma & Rainbow 2005; Meador et al. 2004; Robinson et al. 2006; Whyte et al. 2009).

Because of the different physiological mechanisms for bioaccumulation, detoxification and excretion adopted by different species for different trace elements

at different trophic levels, high concentrations of trace elements transferring to higher trophic levels may not always be observed (Rainbow 2002; Rainbow et al. 2006a). This was seen in the current study with respect to lead and iron. For example, while particulate lead and iron in the diet of the polychaete worms and green-lipped mussels are likely to contribute to body burden in these species, they are not likely to be bioavailable or trophically available to their predators (Kennedy 1986; Marsden et al. 2014; Meador et al. 2004; Rainbow 2002; Rainbow et al. 2006a). In addition, some species are capable of synthesising insoluble metal rich granules, which could be eliminated from the predator species upon intake from their diet, rather than directly absorbed into the body (Rainbow 2002). This could explain why the concentrations of zinc in the potential prey species of the investigated fish were not reflected in muscle trace element levels. Biokinetic parameters also play an important role for controlling the levels of trace elements in different species. This includes the rate of assimilation, ingestion, excretion or elimination/efflux, and growth dilution (Reinfelder et al. 1998; Wang & Rainbow 2008). The higher concentrations of mercury at higher trophic levels are partly due to higher assimilation efficiency compared to a low efflux rate, because of the lipophilic nature of methylmercury (Wang & Rainbow 2008).

5.4.2 TROPHIC TRANSFER POTENTIAL (TTP) AND BIOMAGNIFICATION

Biomagnification through trophic transfer was observed for arsenic, cadmium, copper, mercury and zinc in the studied food chain (Table 5.2). All of these elements increased in concentration in the tissues of the organisms at the higher trophic level, exceeding the levels in the tissues of the prey item (Reinfelder et al. 1998; Wang 2002). Therefore, arsenic, cadmium, copper, mercury and zinc could be considered to have biomagnification potential (at least in the lower trophic levels for cadmium, copper and zinc), although mercury was the only element biomagnified to any appreciable extent in the marine food chain. These results are consistent with other studies (Barwick & Maher 2003; Blackmore & Wang 2004; Hill et al. 1996; Nfon et al. 2009; Storelli et al. 2012; Watras & Bloom 1992; Zhu et al. 2012). Some reviews on trophic transfer of trace elements indicate that there is insufficient evidence for trophic transfer of arsenic, cadmium, copper and lead, but there is compelling evidence for the biomagnification and trophic transfer of mercury, selenium and zinc,

at least in some marine food chains (Gray 2002; Reinfelder et al. 1998; Wang 2002). The main reason for the cadmium, copper and zinc biomagnification observed in this study could be as a result of not depurating the tested animals before analysis to reflect the actual bioaccumulated concentrations in the animal tissues.

The zinc and copper biomagnification observed within the invertebrate species at the bottom of the LH food chain could be due to the efficient accumulation of essential trace elements by these species from the surrounding water, sediments/detritus and micro-organisms. However, the detoxified or the stored forms of the accumulated trace elements within the invertebrates may not be trophically available for efficient assimilation by the predators (Rainbow et al. 2006b; Rainbow & Smith 2010), thus displaying lower concentrations in the higher trophic levels. In fact it is often suggested that invertebrates lack efficient regulatory and detoxification mechanisms that are often present in the higher order vertebrates in the food chain (Bernhard & Andreae 1984; Dallinger 1994), resulting in higher accumulation levels in the invertebrates than the species at the higher trophic levels. Nfon et al. (2009) suggested that the efficient regulatory mechanisms adopted by the higher trophic level vertebrates could be the key characteristic for the lack of biomagnification of essential trace elements at the top of the food chain, a hypothesis supported by the data presented here.

As in this study, biodiminution or trophic dilution of lead and iron has been previously reported in marine and freshwater food chains (Campbell et al. 2005; Nfon et al. 2009; Winterbourn et al. 2000). It is likely that the presence of this phenomenon relates to the chemical form of trace elements present in the aquatic invertebrates. Invertebrates, including bivalves and polychaete worms, are known to detoxify a greater proportion of accumulated trace elements as insoluble metal rich granules, which are less bioavailable to the predators, and which would thus act to reduce the amount of trophically-assimilated trace element (Blackmore & Wang 2004; Nott & Nicolaidou 1990; Rainbow 2002; Rainbow et al. 2006a; Reinfelder et al. 1998; Wang & Rainbow 2010). Additionally, the biodilution of trace elements observed in the organisms at the top of the food chain could also be accounted for by mass dilution (Reinfelder et al. 1998; Wang 2002), where accumulated trace elements are distributed in the muscle tissue, and concentrations are reduced as a consequence of

the large body size of the organism at higher trophic levels (Campbell et al. 2005; Nfon et al. 2009; Reinfelder et al. 1998). Reinfelder et al. (1998) explained trace element dilution in consumers through both mass dilution, but also through the presence of chemical transformations, such as converting inorganic arsenic to arsenobetaine for easier elimination (Zhang et al. 2012). This would result in a reduction in relative burden with increasing trophic level, as observed in the current study.

The mercury results clearly indicated biomagnification across the trophic levels. Fish and crab, at the top of the food chain, contained the highest concentrations, with TTP values greater than one. This is likely due to the predominant storage form of mercury, methylmercury (MeHg), which is not water soluble and is instead lipophilic in nature. Methylmercury characteristically exhibits a high rate of assimilation with a very slow rate of elimination (Blackmore & Wang 2004; Mason et al. 2000; Reinfelder et al. 1998). Therefore, the larger and long-living predator species at the top of the food chain are likely to have mercury biomagnified as a result of accumulation over a longer period of time (Wang 2002; Wang & Fisher 1999).

5.4.3 RISK ASSESSMENT FOR CONSUMPTION OF FISH

The risks associated with consumption of seafood from Lyttelton Harbour are likely to be comparable or lower than other regions of New Zealand. This conclusion results from the observation that trace element concentrations found in the food chain species of LH were generally lower than concentrations in equivalent or identical species in other regions (Brooks & Rumsey 1974; Kennedy 1986; Love et al. 2003; Milne 2006; Peake et al. 2006; Whyte et al. 2009).

Although organs of fish are not commonly consumed by the general population of New Zealand, the relatively higher concentrations of trace elements measured in fish gonads and livers could pose health risks if consumed. Moreover, recreational fishing from LH includes more species of fish than just spotty and banded wrasse, which are not widely consumed. Fish with niches that overlap with these two species include moki, trumpeter, blue cod, blue warehou, grey mullet, grouper, snapper and tarakihi (Leach 2006; Leach et al. 2003; Ministry of Primary Industry

2015). Previous studies measuring trace element concentrations in fish species with similar ecology from the New Zealand coast (Brooks & Rumsey 1974; Love et al. 2003), the Australian coast (Bebbington et al. 1977), the Gulf of Thailand (Ruangwises & Ruangwises 2011) and the Pacific coast of Canada (LeBlanc & Jackson 1973) show levels comparable to those in the current study. Some species such as blue nose (1.3 μg g wet wt^{-1}), ribald (0.94 μg g wet wt^{-1}) and school shark (2.31 μg g wet wt^{-1}) from the New Zealand coast, however, showed higher levels of methylmercury (Love et al. 2003); while hapuku, kingfish and trevally (Brooks & Rumsey 1974) from the New Zealand coast contained over two-fold higher concentraions of copper and zinc than those of this study. This suggests that using the levels of trace elements in spotty and banded wrasse could underestimate true exposure from consumption of LH fish species. Hence, the levels of risks to the community due to trace element intake via fish consumption requires further investigation over a wider range of consumed fish species.

5.5 CONCLUSIONS

Overall, trace elements in the food chain species from the LH sea-fill site displayed higher concentrations than those of PG. The exceptions to this were cadmium in green-lipped mussels and arsenic in green algae, which were higher at PG. These findings are consistent with Chapter 4 of this thesis, which reported significantly higher concentrations of trace elements in seawater and marine sediments for LH than at the PG site (Sections 4.3.2 and 4.3.3).

Fish muscle, crabs and shellfish from LH did not exceed the maximum limits of FSANZ or the EC regulations for any trace elements examined. However, cadmium and mercury concentrations in the liver and kidney are likely to be high enough to cause health risks from regular consumption.

Trophic transfer of trace elements was observed to some extent for both non-essential (arsenic, cadmium and mercury) and essential (zinc and copper) elements. A decreasing trend was observed for iron and lead concentrations along the food chain. Higher concentrations of iron and lead accumulation were observed in the species that

obtain trace elements predominantly from the dissolved phase and sediments, such as green algae and green-lipped mussels, indicating these trace elements are bioavailable in the dissolved phase. Mercury was the only element found to appreciably biomagnify through the entire food chain. More data from species of biota from different trophic levels, covering all the species in the diet of each key organism investigated at each trophic level, are required to confirm the results of the current study. In addition, the biota species could be depurated before trace element analysis to obtain the true bioaccumulated concentrations of trace elements for the trophic transfer studies.

The presented data suggests that the dietary uptake of trace elements is an important route to explain the degree of bioaccumulation in animals such as crustaceans, molluscs and fish. Furthermore, since no previous studies of trace element accumulation in spotty, banded wrasse and cancer crabs exist, the values reported in this study will therefore serve as a baseline for future trace element studies in similar fish species, specifically for risk assessment studies focussed on Lyttelton Harbour.

CHAPTER 6

BASELINE STUDY OF TRACE ELEMENT CONTAMINATION IN SHELLFISH AND A RISK ASSESSMENT FOR CONSUMPTION OF SEAFOOD FROM LYTTELTON HARBOUR OF NEW ZEALAND

6.1 INTRODUCTION

Risk assessment studies have previously been carried out for consumption of New Zealand seafood (NZTDS 2009; Thomson et al. 2008). However, these studies generally focused on commercially-harvested shellfish from well-regulated clean environments, and thus do not reflect the consumption of seafood that may be harvested from contaminated waters. Iwi (Mãori people/tribes) living near coastal areas have ready access to seafood, and often rely on self-harvested wild shellfish

rather than buying seafood from supermarkets (Hay 1996). These wild shellfish collecting sites are not subjected to regulatory control and limited data are available addressing the risk of consuming wild shellfish from New Zealand coastal areas (Whyte et al. 2009).

Baseline studies of trace elements in shellfish have been carried out in a number of different harbours and coastal areas of New Zealand (Brooks & Rumsey 1965; Kennedy 1986; Milne 2006; Nielsen & Nathan 1975; Peake et al. 2006; Whyte et al. 2009). However, limited data are available on trace element concentrations in seafood harvested from Lyttelton Harbour (Sneddon 2011; Sneddon et al. 2010), even though fish and shellfish are regularly collected for consumption from this area (Bolton-Ritchie 2011). As risk assessment for consumption of fish and wild shellfish from Lyttelton Harbour has not previously been undertaken, the potential health risks to the consumers are unknown. There are multiple sources of trace elements to the harbor, including a sea-fill, sewage outfalls, storm water runoff, waste water from the port and the coal stockpile yard, and dry dock discharge (see Chapters 4 and 5). Since coastal populations utilise a number of different seafood sources (Whyte et al. 2009), concentrations of trace elements in three different species of shellfish were investigated in this study.

Target species for this baseline study include New Zealand green-lipped mussels (*Perna canaliculus*), New Zealand cockles (*Austrovenus stutchburyi*) and pipi *(Paphies australis*). These organisms generally do not regulate toxic trace elements in their bodies, leading to accumulation. Therefore, body tissues often reflect the concentrations in the surrounding environment (Rainbow & Phillips 1993; Reinfelder et al. 1998). Green-lipped mussels are found adhering to rocky and hard materials in the intertidal zones, while cockles and pipi are shallow water sediment-burrowing bivalve species. Thus the latter two species may better reflect trace element exposure via the sediment, while the former may better represent trace element uptake through the diet. All of these bivalves take up trace elements from the dissolved phase in solution and via suspended particles in the water column and have high filtration rates that can filter several litres of water every day (Davies & Simkiss 1996).

The two key objectives of this study were to:

- Quantify selected trace elements in three different species of shellfish from the wider Lyttelton Harbour
- Carry out a risk assessment profile for consumption of shellfish harvested from Lyttelton Harbour

6.2 MATERIALS AND METHODS

The laboratory experimental methods to extract trace elements in marine sediments and biota, and their sample preparation for chemical analysis, are provided in Chapter 2.

6.2.1 SAMPLE COLLECTION

Green-lipped mussels (*Perna canaliculus*), cockles (*Austrovenus stutchburyi*) and pipi (*Paphies australis*) were collected for this study (10th March - 19th April 2012) from 7 sites across the wider Lyttelton Harbour (Figure 6.1), depending on their availability. Green-lipped mussels (n = 10 each site, 74 - 108 mm shell length, 6 sites) and cockles (n = 10 each site, 32 - 51 mm shell length, 6 sites) were collected by hand at low tide. Pipi (n = 10 each site, 47 - 71 mm shell length, 3 sites) were collected by finger dredging or raking though the sand of the sampling sites at very low tide (Figure 6.2A). The shellfish were placed in zip-lock plastic bags. Triplicate sediment samples were also collected (in acid-cleaned plastic jars) from each shellfish collection site. The samples were transported to the laboratory on ice (Chandurvelan et al. 2013). The shellfish samples were collected from Corsair Bay (CO), Cass Bay (CA), Rapaki (RP), Sandy Bay (SB), Purau Bay (PR), Port Levy (PL) and Pigeon Bay (PG) (Figure 6.1).

The shellfish were cleaned by scraping any adhering epibionts off the shells, and dried with paper towels before any measurements were recorded. The shellfish were not depurated prior to dissection to reflect consumption practices. The whole body soft tissues were collected in pre-cleaned and pre-weighed polyethylene vials.

Wet tissue measurements were recorded before storage at -20^{o}C prior to freeze drying. Sediment samples were oven-dried and treated as described in Section 2.4.1.1 for analysis. Shellfish samples were collected under the Ministry of Primary Industries permit provided to the School of Biological Sciences of the University of Canterbury for research purposes.

Figure 6.1: Shellfish collected sites in wider Lyttelton Harbour: CA- Cass Bay, CO- Corsair Bay, RP- Rapaki, SB-Sandy Beach, PR- Purau Bay, PL- Port Levy, PG- Pigeon Bay. Map adapted from Google Earth

Figure 6.2: Pipi collection at Rapaki (A), and mussel and cockle beds in Sandy Beach (B)

6.2.2 RISK ASSESSMENT FOR CONSUMPTION OF SHELLFISH

Trace element exposures were estimated using data provided for the age-gender cohort of 25+ yr adults (male – 82 kg; female – 70 kg), children of 5-6 yr (32 kg) and toddlers of 1-3 yr (13 kg) at the rates of fish and other seafood consumption for the general population of New Zealand provided in the 2009 New Zealand Total Diet Study (NZTDS 2009) as described in Chapter 5 (Section 5.2.3). Trace element data of fish muscle from Chapter 5 were used for the risk assessment calculations to obtain the EWI values for consumption of fish and shellfish combined. The rate of total shellfish consumption for a male adult was 7 g per day (49 g per week), and for a female adult was 3 g per day (21 g per week). Children and toddlers were assumed not to consume shellfish for the NZTDS. A higher shellfish consumption rate (2849 g/week; 407 g per person per day) targeted for higher shellfish consumers was provided in a report on New Zealand shellfish harvesting and consumption (King & Lake 2013) was used to calculate an exposure scenario for heavily shellfish-dependent populations. This ingestion rate was more relevant to those with ready access to shellfish harvesting locations, such as people living near the coast.

Although shellfish retain some mercury in an inorganic form (Hight & Cheng 2006), for the purposes of the risk assessment calculations, 100% of total mercury in shellfish was assumed to be methylmercury. The highest mean concentrations of the four toxic trace elements (arsenic, cadmium, mercury and lead) measured in any shellfish among the sampling sites from the wider Lyttelton Harbour were used for the risk assessment. In addition, the maximum individual concentration of each trace element from any shellfish in any one site was also used to estimate the worst case scenario for the two consumption rates. The trace element exposure doses were calculated as described in Section 3.2.2.4.

6.2.3 METAL POLLUTION INDEX (MPI)

The metal pollution index (MPI) was used to compare trace element contents at the shellfish sampling sites. The MPI values were calculated using Equation 4.1 in Section 4.2.5, using dry weights.

6.2.4 BIOSEDIMENT ACCUMULATION FACTOR (BSAF)

The biosediment accumulation factor (BSAF) was calculated to evaluate trace element bioaccumulation in different species of shellfish from the associated sediments. The BSAF was calculated using Equation 6.1 (Szefer et al. 1999),

$$BSAF = {C_b}/{C_S} \qquad \textbf{Equation 6.1}$$

where C_b is the mean concentration of the trace element in the biota (shellfish) in dry weight and C_s is the mean concentration of the same trace element in the associated sediment in dry weight.

6.2.5 STATISTICAL ANALYSIS

All statistical analyses were carried out in R© (Version 2.15.3). Statistical analysis was performed only for the sample sets where more than 50% of the samples had levels above the limit of quantification (LOQ). For analytes detected but below the LOQ, a value of half the LOQ was used in the statistical analysis. All duplicate measurements were averaged before inclusion in the statistical analysis.

All data were checked for normality by plotting probability plots. Where necessary, data were log transformed to meet assumptions of normality before analysis. Significant differences ($p < 0.05$) at the 95% confidence level for trace element concentrations in shellfish species between the sites were determined by using single factor ANOVA tests followed by Tukeys HSD test ($p < 0.05$). Pearson's correlation coefficients were used to analyse the relationships between the trace element concentrations in the shellfish and the associated sediments. Pearson's correlation coefficients were also used to analyse relationships between trace elements within the same species and within the sediments.

6.3 RESULTS

6.3.1 ANALYTICAL METHOD PERFORMANCE

Standard reference materials of mussel tissue (SRM-2976, EVISA) and fish

protein (DORM-3) were digested along with shellfish samples. A marine sediment certified reference material (SRM-2702-NIST) was also digested along with the sediments of the shellfish collection sites. The percentage recoveries of all elements in the standard reference mussel and fish protein (DORM-3) were greater than 90%, with the exception of lead in DORM-3, which was 34.9% (see Section 2.4.3.2 for detail). The percentage recoveries of trace elements in the certified reference sediments ranged from 98.8 to 102.4% (Table 6.1).

Table 6.1: Mean percentage recoveries ± standard error for standard reference materials

	Mean % recoveries of trace elements for standard reference materials		
Analytes	Mussel (n = 6)	Fish protein (n = 6)	Sediment (n = 2)
Arsenic	116.4 ± 4.8	97.6 ± 6.2	94.9 ± 0.8
Cadmium	104.7 ± 2.3	101.8 ± 2.6	102.4 ± 0.8
Copper	90.1 ± 4.4	90.2± 2.2	90.1 ± 0.2
Iron	103.9 ± 6.4	96.0± 2.3	92.9 ± 2.8
Mercury	91.7 ± 11.2	90.6 ± 8.3	89.8 ± 3.2
Lead	97.1 ± 5.2	36.4 ± 4.0	90.2 ± 0.9
Zinc	101.7 ± 6.4	97.0 ± 8.6	91.7 ± 5.2

The percentage differences of the 10th sample duplicate digests were less than 10%; while the duplicate dilutions were within 7%. The percentage recoveries of spiked elements in the samples ranged between 88-115%.

6.3.2 TRACE ELEMENT CONCENTRATIONS IN SHELLFISH

The trace elements analysed in three shellfish species and associated sediments collected around the wider Lyttelton Harbour were arsenic, cadmium, copper, iron, lead, mercury and zinc. Mercury was less than the LOQ in sediments. The trace element concentrations (mean values in µg g wet wt^{-1}) measured in the shellfish and associated sediments (mean value µg g dry wt^{-1}) from the wider Lyttelton Harbour are presented in Table 6.2. This table also includes the MPI values calculated for the three shellfish species and sediments for each site; and the BSAF values calculated for each of the trace elements (except mercury, which was <LOQ) in the three shellfish species. Trace element concentrations in sediments were not compared in detail to other studies, as the main purpose of sediment analysis was to determine relationships with the concentrations of trace elements found in the

shellfish. Trace element concentrations in shellfish were presented in wet weight unless specified. Abbreviations for site names are provided in Table 6.2.

In general, mercury, iron and zinc accumulation followed a similar pattern (green-lipped mussels > cockles > pipi), while cadmium and lead followed similar trends (green-lipped mussels > pipi > cockles) among the three shellfish species. Arsenic accumulation was highest in cockles followed by green-lipped mussels and then pipi; while copper accumulation was highest in pipi followed by cockles and then green-lipped mussels. Specific analysis of the data is detailed below.

6.3.2.1 ARSENIC

The mean total arsenic concentrations measured in the shellfish species ranged from 1.66 to 2.45 µg g^{-1}, from 4.36 to 9.93 µg g^{-1} and from 1.99 to 2.86 µg g^{-1} for green-lipped mussels, cockles and pipi respectively. Total arsenic concentrations in cockles were significantly higher than those of green-lipped mussels and pipi, but there was no significant difference between these latter two species (cockles > green-lipped mussels = pipi). For green-lipped mussels, the total arsenic concentrations from sites PL, PG and SB were significantly higher ($p < 0.05$) than those of the RP and PR sites, and mussels from site PL were significantly higher in arsenic than those from the SB site. For cockles, total arsenic concentrations of the PG and SB sites were significantly higher than those of the CA, RP and PL sites. In pipi, total arsenic concentrations of the RP site were significantly higher than those of the CO and PL sites. The mean concentrations of total arsenic in the sediments at different sites ranged from 5.05 to 16.10 µg g dry wt^{-1}. The arsenic content in the sediments followed the order: PL > PG > CO = SB = PR > RP > CA.

6.3.2.2 CADMIUM

The mean concentrations of cadmium measured in the shellfish species ranged from 0.055 to 0.094 µg g^{-1}, from 0.029 to 0.052 µg g^{-1} and from 0.048 to 0.072 µg g^{-1} for green-lipped mussels, cockles and pipi respectively. Cadmium concentrations therefore followed the order: green-lipped mussels > pipi > cockles. For green-lipped mussels, cadmium concentrations in samples from the PG site were significantly higher than those of the RP and PR sites, and concentrations in the green-lipped mussels of PL site were significantly higher than those of the RP site. For cockles,

cadmium concentrations in the PG site were significantly higher than those of the CA, RP and PL sites. Cadmium concentrations in pipi from the PL site were significantly higher than those of CO and RP. Cadmium concentrations in sediments ranged from 0.019 to 0.069 µg g dry wt $^{-1}$, and followed the order: PG > PL > SB = PR > RP > CA = CO.

6.3.2.3 COPPER

The mean concentrations of copper in the shellfish species ranged from 0.71 to 0.98 µg g^{-1}, from 0.88 to 1.22 µg g^{-1} and from 0.87 to 1.78 µg g^{-1} for green-lipped mussels, cockles and pipi respectively. Copper concentrations followed the order: pipi > cockles > green-lipped mussels. For green-lipped mussels, copper concentrations in the SB, RP and PL sites were significantly higher than those of the PG site, and also copper concentrations at SB were significantly higher than at PR. Concentrations of copper in cockles from the PR site were significantly higher than those of the PG site, and there were no significant differences between any other sites for this species. For pipi, copper concentrations in the CO site were significantly higher than those of the PL and RP sites. The mean copper concentrations in sediments ranged from 4.26 to 17.44 µg g dry wt^{-1}. Copper concentrations at the different sites followed the order: PL > PG > SB > CO > PR > RP > CA.

6.3.2.4 IRON

The mean iron concentrations in shellfish species from different sites ranged from 150 to 212 µg g^{-1}, from 41 to 119 µg g^{-1} and from 68 to 76 µg g^{-1} for green-lipped mussels, cockles and pipi respectively. Statistically, the iron contents of the shellfish species were in the order: green-lipped mussels > cockles = pipi. The iron concentrations in green-lipped mussels from the SB site were significantly higher than those of the PG site; but there were no other significant differences in green-lipped mussel iron between other sites. For cockles, the iron concentrations in the animals collected from the RP site were significantly higher than those of the PG, PL and PR sites. Cockles from the SB site were significantly higher in iron than cockles from the PL and PR sites. There were no significant differences in iron concentrations between pipi with respect to site. Sediment iron concentrations between the sampling sites ranged from 10973 to 45898 µg g dry wt^{-1}. Concentrations of iron in sediments

followed the order: PL = PG > SB > PR > RP = CO = CA.

Table 6.2: Trace element concentrations (mean) in shellfish and harbour sediments, metal pollution index (MPI) and mean biota-sediment accumulation factor (BSAF)

Sample site	As	Cd	Cu	Fe	Hg	Pb	Zn	MPI
Green mussel (µg g wet wt^{-1})								
Sandy Beach (SB)	2.14	0.068	0.98	211.70	0.050	0.41	21.82	7.60
Rapaki (RP)	1.66	0.055	0.90	155.82	0.038	0.32	15.31	5.96
Purau (PR)	1.75	0.063	0.78	172.02	0.039	0.27	17.32	6.04
Port Levy (PL)	2.45	0.088	0.90	152.93	0.019	0.16	17.57	5.55
Pigeon Bay (PG)	2.35	0.094	0.71	150.37	0.021	0.15	14.67	5.28
mean BSAF (dry wt based)	**1.22**	**9.24**	**0.53**	**0.04**		**0.09**	**1.48**	
Cockle (µg g wet wt^{-1})								
Cass Bay (CA)	6.03	0.029	1.19	81.86	0.019	0.12	10.98	4.88
Rapaki (RP)	6.31	0.030	1.04	118.94	0.020	0.13	11.94	5.26
Sandy Beach (SB)	9.93	0.036	0.99	109.43	0.042	0.12	11.16	6.30
Purau (PR)	7.49	0.031	1.22	45.59	0.035	0.05	11.29	4.57
Port Levy (PL)	4.36	0.035	0.92	40.73	0.014	0.03	10.96	3.33
Pigeon Bay (PG)	9.71	0.052	0.88	64.12	0.022	0.05	12.62	4.79
BSAF (dry wt based)	**5.27**	**5.44**	**0.84**	**0.025**		**0.031**	**1.15**	
Pipi* (µg g wet wt^{-1})								
Corsair Bay (CO)	2.13	0.048	1.78	67.51	0.025	0.24	11.35	4.64
Rapaki (RP)	2.86	0.047	1.11	73.53	0.028	0.24	11.95	4.71
Port Levy (PL)	1.99	0.072	0.87	76.06	0.015	0.05	13.65	3.42
BSAF (dry wt based)	**1.34**	**9.28**	**0.82**	**0.02**		**0.05**	**1.12**	
Harbour sediment (µg g dry wt^{-1})								
Corsair Bay(CO)	8.16	0.019	7.37	11358.92	<LOQ	18.56	51.29	15.35
Cass Bay (CA)	5.05	0.020	4.26	10973.25	<LOQ	13.31	37.72	11.55
Rapaki (RP)	6.24	0.025	5.12	11443.32	<LOQ	13.51	35.30	12.31
Sandy Beach (SB)	7.69	0.040	11.87	25551.66	<LOQ	20.09	67.02	22.38
Purau (PR)	7.37	0.036	5.67	19347.52	<LOQ	14.46	55.03	17.18
Port Levy (PL)	16.10	0.047	17.44	45897.50	<LOQ	14.15	96.78	30.97
Pigeon Bay (PG)	10.73	0.069	13.33	41629.16	<LOQ	12.00	92.20	27.80
ANZECC, ISQG-low value	**20**	**1.5**	**65**	******	**0.15**	**50**	**200**	

***Pipi were found only in three sites; ** no ISQG value provided; LOQ- Limit of quantification**

6.3.2.5 MERCURY

The mean concentrations of mercury in the shellfish species ranged from 0.019 to 0.050 µg g^{-1}, from 0.014 to 0.040 µg g^{-1} and from 0.015 to 0.025 µg g^{-1} for green-lipped mussels, cockles and pipi respectively. Mercury concentrations in green-lipped mussels were significantly higher than those of cockles and pipi (green-lipped mussels > cockles = pipi). The concentrations of mercury in green-lipped mussels from different sites followed the order: SB > PR = PR > PG = PL. For cockles, mercury concentrations at the site SB were significantly higher ($p < 0.05$) than those of sites PG, RP, CA and PL. The concentrations of mercury in cockles from site PR were also significantly higher than those cockles from PL and CA. Mercury concentrations in pipi followed the order: CO = RP > PL. Mercury was not detected in any of the sediments.

6.3.2.6 LEAD

The mean lead concentrations for green-lipped mussels, cockles and pipi ranged from 0.15 to 0.41 µg g^{-1}, from 0.03 to 0.13 µg g^{-1} and from 0.05 to 0.24 µg g^{-1} respectively. Lead concentrations measured in the green-lipped mussels from site SB (0.41 µg g^{-1}) displayed the highest mean concentrations, and cockles from site PL (0.03 µg g^{-1}) displayed the lowest mean concentrations. The order of lead concentrations between the shellfish species was green-lipped mussels > pipi > cockles. Lead concentrations in green-lipped mussels followed the order: SB > RP = PR > PL = PG. Lead concentrations in cockles from the sites RP and SB were significantly higher than those of PR, PG and PL, and concentrations in cockles from site CA were significantly higher than those of sites PG and PL. For pipi, lead concentrations in sites CO and RP were significantly higher than those for pipi sampled from site PL. Mean concentrations of lead in the sediments ranged from 12.00 to 20.09 µg g dry wt^{-1}. Lead content in the sediment of site SB was significantly higher than that of all the other sites with the exception of site CO, while lead in site CO was also significantly higher than that of all the other sites. Concentrations of lead in sediments of the PR site were significantly higher than those of the PG site.

6.3.2.7 ZINC

The mean concentrations of zinc for shellfish species collected from different sampling sites ranged from 14.67 to 21.82 µg g^{-1}, from 10.96 to 12.62 µg g^{-1} and from 11.35 to 13.65 µg g^{-1} for green-lipped mussels, cockles and pipi respectively. Zinc concentrations followed the order: green-lipped mussels > cockles = pipi. The zinc content in green-lipped mussels followed the order: SB > PL = PR = RP = PG. For cockles, zinc concentrations from site PG were significantly higher ($p < 0.05$) than those of sites CA and PL, while zinc concentrations were not significantly different between any of the sampling sites for pipi (PL = RP = CO). Mean concentrations of sediment zinc at different shellfish sites ranged from 35.30 to 96.78 µg g dry wt^{-1}. Zinc concentrations in sites PL and PG were significantly higher ($p < 0.05$) than those of the SB, PR, CO, CA and RP sites. Concentrations of zinc from site SB were significantly higher than those of sites CO, CA and RP. Zinc contents from sites PR and CO were significantly higher than those of the CA and RP sites.

6.3.2.8 CORRELATION ANALYSIS OF TRACE ELEMENTS IN SHELLFISH AND SEDIMENT

Pearson's correlations analyses were carried out to investigate the relationships between concentrations of trace elements in shellfish and the associated sediments. The correlation coefficients and the respective *p*-values are presented in Table 6.3. This relationship is also shown in Appendix D1, by best fit regression lines for each species of shellfish for each trace element.

Table 6.3: Pearson correlation coefficients (r) and *p*-values for correlations of trace element concentrations between the shellfish and associated sediments

	Mussels (n = 50)		**Cockles (n = 60)**		**Pipi (n = 30)**	
	r	*p*-value	r	*p*-value	r	*p*-value
As	0.844	0.072	-0.288	0.580	-0.752	0.458
Cd	**0.928**	**0.023**	**0.937**	**0.006**	0.978	0.133
Cu	0.087	0.889	**-0.845**	**0.034**	-0.573	0.611
Fe	-0.302	0.621	-0.579	0.229	0.729	0.480
Pb	0.805	0.100	0.342	0.508	0.431	0.716
Zn	0.014	0.982	0.184	0.728	0.874	0.322

Values in bold represent statistical significance ($p < 0.05$)

Cadmium concentrations in green-lipped mussels and cockles were significantly positively correlated ($p < 0.05$) with the cadmium concentrations in sediments. Copper concentrations in cockles were significantly ($p < 0.05$) negatively correlated with copper concentrations in sediments. There were no significant correlations for lead, iron and zinc.

Correlation analysis was also carried out for trace elements within each species of shellfish and within sediments (see Appendix D2). Iron-zinc, cadmium–arsenic, and lead-mercury pairs were significantly positively correlated in green-lipped mussels. For cockles, lead-iron was significantly positively correlated; while copper-iron and lead-cadmium pairs were significantly negatively correlated in pipi. Pearson correlation coefficients and *p*-values for trace elements within the sediments indicated that iron, copper, zinc, arsenic and cadmium were significantly positively correlated.

6.3.2.9 METAL POLLUTION INDEX (MPI)

The MPI values calculated from the green-lipped mussels (Table 6.2) indicated that Sandy Beach (SB) was the site with the most contaminated mussels, followed by Purau (PR) and Rapaki (RP). The MPI values obtained from cockles also indicated that SB site had the highest contamination load followed by the Port Levy (PL) and RP sites. The values obtained from pipi were not taken into account, because pipi were only available from a limited number of sites. MPI values calculated from sediments indicated that the PL site contained the most highly contaminated sediments, followed by PG and SB.

6.3.2.10 BIOSEDIMENT ACCUMULATION FACTORS (BSAF)

The bio-sediment accumulation factor (BSAF) calculated from the green-lipped mussels followed the order: Cd > Zn ≥ As > Cu > Pb > Fe. The BSAF value obtained from cockles followed the order: Cd > As > Zn > Cu > Pb ≥ Fe. For pipi the BSAF values followed the order: Cd > As > Zn > Cu > Pb > Fe. Cadmium was placed at the top of the BSAF values calculated for all three species of shellfish, followed by arsenic and zinc. The lowest BSAF values were obtained for lead and iron for all species.

6.3.2.11 RISK ASSESSMENT FOR CONSUMPTION OF FISH AND SHELLFISH

Estimated weekly intakes (EWI) of inorganic arsenic, cadmium, mercury and lead were calculated for fish and shellfish consumption. The total EWI values (fish + shellfish) were calculated by adding the individual EWI values of fish and shellfish for people who consume both species, using the highest mean concentrations measured in each food group. The EWI values for fish were calculated using the concentration data of fish species described in Chapter 5.

The provisional tolerable weekly intake (PTWI) values for inorganic arsenic, cadmium, mercury or lead were not exceeded at rates of fish and shellfish consumption by adults of the general population of New Zealand. However, the PTWI value for mercury was exceeded for toddlers, using the average fish consumption rates provided in the NZTDS at the 95^{th} percentile concentration, and maximum mercury concentration values in fish (Table 6.4). Also, at the highest rate (2849 g/week) of shellfish consumption, adults of both gender groups exceeded the PTWI value (Table 6.4) for inorganic arsenic and mercury. Similarly, adult females exceeded the PTWI value for cadmium at high shellfish consumption rates (2849 g/week) (King & Lake 2013).

Table 6.4: Estimated weekly intake (EWI) of trace elements at different consumption rates by different age-gender cohorts at different concentrations of trace elements in edible tissues

		Consumption rate (g/week)	EWI of trace elements (µg/kg BW/week)			
			Inorg. As	Cd	Hg	Pb
	WHO / JECFA PTWI values		**21**	**5.6**	**1.6**	**25**
Fish consumption	**82 kg male**	245				
	Mean conc.		0.82	-	0.83	-
	95th percentile conc.		1.27	-	1.10	-
	Max conc.		1.58	-	1.13	-
	70 kg female	168				
	Mean conc.		0.66	-	0.67	-
	95th percentile conc.		1.02	-	0.88	-
	Max conc.		1.27	-	0.91	-
	32 kg child					
	Mean conc.	91	078	-	0.79	-
	95th percentile conc.		1.21	-	1.04	-
	Max conc.		1.51	-	1.08	-
	13 kg toddler	63				
	Mean conc.		1.33	-	1.35	-
	95th percentile conc.		2.06	-	**1.78**	-
	Max conc.		2.57	-	**1.84**	-
Shellfish consumption	**82 kg male**					
	Highest mean conc.	49	0.59	0.05	0.03	0.25
	Highest individual conc.	49	0.83	0.09	0.04	0.31
	Highest mean conc.	2849	**34.40**	3.13	**1.74**	14.25
	Highest individual conc.	2849	**48.43**	5.21	**2.43**	18.07
	70 kg female					
	Highest mean conc.	21	0.30	0.03	0.02	0.12
	Highest individual conc.	21	0.42	0.05	0.02	0.16
	Highest mean conc.	2849	**40.29**	3.66	**2.04**	16.69
	Highest individual conc.	2849	**56.74**	**6.11**	**2.85**	21.16
Fish & shellfish consumption	**82 kg male**					
	Highest mean conc.-fish	245	0.82	0.00	0.83	0.00
	Highest mean conc.-shellfish	49	0.59	0.05	0.03	0.25
	Total EWI		*1.41*	*0.05*	*0.86*	*0.25*
	70 kg female					
	Highest mean conc.-fish	168	0.66	0.00	0.67	0.00
	Highest mean conc.-shellfish	21	0.30	0.03	0.02	0.12
	Total EWI		*0.95*	*0.03*	*0.68*	*0.12*

Values in bold exceed the PTWI value. **(WHO/ JECFA)-Joint Expert Committee for Food Additives**

6.4 DISCUSSION

Iron was the most abundant trace element in the three shellfish species investigated. Thereafter, the trace elements found at highest levels were zinc and then arsenic, followed by copper and then lead. Cadmium and mercury were found at very low concentrations among all three species. These findings are in agreement with other bivalve shellfish studies reported elsewhere (Kulikova et al. 1985; Marsden et al. 2014; Usero et al. 1997). Similar trends of trace element concentrations were also reported in green-lipped mussels and blue mussels (*Mytilus edulis*) from Wellington Harbour (Kennedy 1986). However the study of Kennedy (1986) also reported that green-lipped mussels from one site of Wellington Harbour contained higher mean concentrations of lead than that of arsenic and copper, suggesting the levels of trace elements found in the shellfish are also dependent on the level of site contamination, as evident in this study.

In general, mean trace element concentrations (µg g dry wt^{-1}) in sediments followed the order: iron (23743) > zinc (62.19) > lead (15.15) > copper (9.29) > arsenic (8.76) > cadmium (0.04). The highest mean concentrations of arsenic, copper, iron and zinc were measured in PL site sediments, while the PG site displayed the highest concentrations of cadmium, and site SB contained the highest mean concentrations of lead. Similar to shellfish, the most abundant elements in sediments were iron and zinc. The trace element concentration trends observed in sediments of this study were in agreement with other sediment studies (Leivuori et al. 2000). It is common to observe higher concentrations of zinc and iron than other elements as these are naturally more abundant (Haynes 2014; Usero et al. 2005).

Trace element concentrations in the biota samples were compared with the maximum allowable levels (ML) of the Food Standards Australia New Zealand (FSANZ) standard code, and maximum allowable values of the European Commission (EC) regulations. These values are presented in Table 1.3, Chapter 1. None of the shellfish species in any site exceeded the maximum allowable value for inorganic arsenic, cadmium, mercury or lead in FSANZ or EC regulations. Also, no shellfish sample exceeded maximum allowable levels for copper and zinc in the "other trace element standards" provided by the FAO for New Zealand and South

Australia (Table 1.3, Chapter 1). However, cockle samples from the PG site (0.97 µg g^{-1} inorganic As) and the SB site (0.99 µg g^{-1} inorganic As) were very close to the maximum allowable value of 1 µg g^{-1} for inorganic arsenic in molluscs.

Comparisons of trace element concentrations in the shellfish-associated sediments to the ANZECC guidelines (ANZECC 2000) showed that all the investigated trace element concentrations were well below the recommended ISQG-low trigger values recommended for contaminants in marine sediments (Table 6.2). More detail of the ANZECC guidelines, and the ISQG-low and ISQG-high values are provided in Chapter 4.

There were no significant correlations between lead, iron and zinc in any shellfish species and the associated sediments. However, cadmium (positive correlation) and copper (negative correlation) were significantly correlated. The general lack of correlation could be explained by the small number of samples, by the lack of sediment trace element bioavailability, or by the fact that these organisms were able to regulate trace element burdens, especially of essential elements for which specific pathways of uptake and/or elimination are present (e.g. zinc, copper, iron). Alternatively, the trace element levels in the sediments were not at a sufficiently high concentration to result in significant biotic accumulation (Usero et al. 2005).

The high MPI measured in the shellfish and sediments from Sandy Bay (SB) was not a surprise considering the presence of a sewage outfall in Governors Bay, in close proximity to the SB sampling site. The general trend of trace element contamination observed from the MPI values of the shellfish suggested that SB site contained the highest bioavailable fraction of trace elements followed by the Purau (PR) and Rapaki (RP) sites, although the total amounts of trace elements (both biologically available and unavailable) were higher in the Port Levy (PL) and Pigeon Bay (PG) sites as observed from the MPI values of sediments. The iron concentrations measured in sediments from PL and PG sites were nearly twice those of other sediments in this study, likely driving these elevated MPI ratings. In addition, PL and PG also contained comparatively higher concentrations of arsenic, copper and zinc. The sediment iron concentrations of Banks Peninsula (Lyttelton Harbour and Pigeon Bay, Chapter 4) suggests that the higher concentration of iron observed in the more remote sites (PL and PG) were likely due to naturally elevated geological levels, as

volcanic areas are found to have elevated iron levels (Denton & Morrison 2009). The other sampling sites were also located in Banks Peninsula (inner Lyttelton Harbour), but had lower iron concentrations. This could be due to covering of the iron-rich sediment layer with other materials, a consequence of anthropogenic activities associated with urban development at inner Lyttelton Harbour sites.

The mean BSAF values obtained for the three species of this study suggest that, in general, the green-lipped mussels had a greater capacity for trace element bioaccumulation than cockles or pipi, with the exception of arsenic and copper for cockles (Table 6.2). Cadmium was placed at the top of the BSAF values calculated for all three species of shellfish, suggested that cadmium has the highest efficiency for bioaccumulation from sediments, followed by arsenic and zinc, while lead and iron were taken up with the least efficiency from the sediments. This trend was common to all three species investigated in this study. The lowest BSAF value, derived for iron, could be explained by efficient homeostatic mechanisms for regulating essential elements, including iron, in bivalves. The relative ability to sequester trace elements from sediments will vary among different shellfish species depending on a number of factors, including behaviour (i.e. burrowing versus non-burrowing species), and diet (Gundacker 2000).

The provisional tolerable weekly intake (PTWI) values for inorganic arsenic, cadmium, mercury and lead were not exceeded at rates of fish and shellfish consumption by adults of the general population of New Zealand. Hence there is little risk involved in consumption of fish and shellfish harvested from Lyttelton Harbour for adults assuming the PTWI values are protective. However, adult females can exceed the PTWI value for cadmium at high shellfish consumption rates (2849 g/week). This could be of particular concern for New Zealanders such as Mãori and Pacific Islanders, as seafood can comprise a major proportion of their diet (Hay 1996; Russel et al. 1999). In the 2002 National Children's Nutrition Survey (Parnell et al. 2003), fish and seafood were shown to contribute twice the proportion of protein for Pacific ethnic group children than for New Zealand European and other ethnic group children.

Table 6.5: Amount of shellfish required to be consumed by a 70 kg adult per week to exceed the WHO / JECFA PTWI values; and comparisons with the results from Bay of Islands / NZ

Element	Concentrations (µg g wet wt^{-1})			Amount of shellfish flesh (kg) per week required to exceed PTWI values		
	Max. mean for any 1 site	Max. individual value	Min. mean for any 1 site	Based on max. mean	Based on max. individual value	Based on min. mean
As*	0.99 (SB)	1.39 (SB)	0.17 (RP)	1.49	1.06	8.65
Cd	0.09 (PG)	0.15 (PG)	0.03 (CA)	4.36	2.61	13.07
Hg	0.05 (SB)	0.07 (SB)	0.01 (PL)	2.24	1.60	11.20
Pb	0.41 (SB)	0.41 (SB)	0.03 (PL)	4.27	4.27	28.33
Concentrations of trace elements in green-lipped mussels from Bay of Islands, NZ (Whyte et al. 2009) for comparison						
As*	0.3	0.37	0.16	4.9	3.97	9.19
Cd	0.75	1.37	0.07	0.52	0.29	5.6
Hg	0.06	0.08	0.05	1.87	1.4	2.24
Pb	0.1	0.15	0.03	17.5	11.67	58.33

***Inorganic arsenic; PTWI-provisional tolerable weekly intake**

Previous risk assessments of shellfish consumption (Whyte et al. 2009), indicated that a comparatively large quantity of green-lipped mussels from the Bay of Islands would need to be consumed to exceed PTWI values for inorganic arsenic (>4.9 kg) and lead (>17 kg) (Table 6.5). Even larger quantities of shellfish from Lyttelton Harbour would be required to exceed the PTWI values for cadmium and mercury (Table 6.5). The higher accumulation of arsenic by cockles, compared to green-lipped mussels and pipi, would certainly pose higher health risks to the heavy consumers of cockles from Lyttelton Harbour. The maximum and minimum values of trace elements for calculating the EWI were obtained from among the three shellfish species rather than taking one species as in the Whyte et al. (2009) study.

Only limited data are available on arsenic concentrations in green-lipped mussels and other bivalves in New Zealand (Robinson et al. 1995; Whyte et al. 2009). In general, previous studies on the trace element concentrations in shellfish species were comparable to this study (Tables 6.6 - 6.12).

Table 6.6: Comparison of total arsenic concentrations in bivalve shellfish with existing literature

		Arsenic		
Species	Locations / description	Mean (range) µg g^{-1}		Reference
		Wet wt	Dry wt	
Mussel				
Perna canaliculus	Lyttelton Harbour /NZ	2.07 (1.66 - 2.45)	10.92 (8.99 - 13.14)	This study
Perna canaliculus	Bay of Islands / NZ	2.11 (1.56 - 2.97)		(Whyte et al. 2009)
Perna canaliculus	Wellington Harbour		10.5	(Kennedy 1986)
Mytilus edulis	Wellington Harbour		(7.2 - 13.6)	(Kennedy 1986)
Mytilus edulis aoteanus	Mouth of Waikato River /NZ	1.10 ± 0.75		(Robinson et al. 1995)
Perna viridis	Thailand coast	2.35 (1.07- 4.30)		(Ruangwises & Ruangwises 2011)
Perna viridis	Singapore coast		(13 - 32)	(Bayen et al. 2004)
Cockle				
Austrovenus stutchburyi	Lyttelton Harbour /NZ	7.31(4.36 - 9.93)	40.57 (25.93 - 55.04)	This study
Austrovenus stutchburyi	Mouth of Waikato River /NZ	1.24 ± 0.39		(Robinson et al. 1995)
Anadara granosa	Thailand coast	32.2 (30.5 - 35.5)		(Ruangwises & Ruangwises 2011)
Austrovenus stutchburyi	Avon-Heathcote Estuary / NZ		(10.2 - 46.3)	(Marsden et al. 2014)
Pipi				
Paphies australis	Lyttelton Harbour / NZ	2.33 (1.99 - 2.86)	11.10 (9.46 - 13.61)	This study
Paphies australis	Mouth of Waikato River / NZ	1.01 ± 0.21		(Robinson et al. 1995)

Table 6.7: Comparison of cadmium concentrations in bivalve shellfish with existing literature

		Cadmium		
Species	Locations / description	Mean (range) µg g^{-1}		Reference
		Wet wt	Dry wt	
Mussel				
Perna canaliculus	Lyttelton Harbour / NZ	0.07 (0.055 - 0.094)	0.38 (0.29 - 0.49)	This study
Perna canaliculus	Bay of Islands / NZ	0.26 (0.07 - 0.75)		(Whyte et al. 2009)
Perna canaliculus	All around NZ	0.30 (0.10 - 1.00)		(Nielsen & Nathan 1975)
Mytilus galloprovincialis	South and Southwest coast of Wellington / NZ	(0.10 - 0.20)		(Milne 2006)
Cockle				
Austrovenus stutchburyi	Lyttelton Harbour / NZ	0.04 (0.030 - 0.052)	0.20 (0.16 - 0.30)	This study
Austrovenus stutchburyi	South and Southwest coast of Wellington / NZ	0.01 - 0.03)		(Milne 2006)
Austrovenus stutchburyi	Avon-Heathcote Estuary / NZ		(0.21 - 0.42)	(Marsden et al. 2014)
Austrovenus stutchburyi	All around NZ	0.19		(Nielsen & Nathan 1975)
Pipi				
Paphies australis	Lyttelton Harbour /NZ	0.06 (0.047 - 0.072)	0.26 (0.22 - 0.35)	This study
Paphies australis	All around NZ	0.13 (0.12 - 0.14)		(Nielsen & Nathan 1975)
Paphies subtriangulata	South and Southwest coast of Wellington / NZ	(0.07 - 0.08)		(Milne 2006)

Table 6.8: Comparison of mercury concentrations in bivalve shellfish with existing literature

		Mercury		
Species	Locations / description	Mean (range) µg g^{-1}		Reference
		Wet wt	Dry wt	
Mussel				
Perna canaliculus	Lyttelton Harbour /NZ	0.03 (0.02 - 0.05)	0.17 (0.10 - 0.26)	This study
Perna canaliculus	Bay of Islands / NZ	0.03		(Whyte et al. 2009)
Perna canaliculus	All around NZ	0.09 (0.04 - 0.19)		(Nielsen & Nathan 1975)
Mytilus galloprovincialis	South and Southwest coast of Wellington / NZ	(0.006 - 0.012)		(Milne 2006)
Cockle				
Austrovenus stutchburyi	Lyttelton Harbour /NZ	0.03 (0.01 - 0.04)	0.14 (0.10 - 0.23)	This study
Austrovenus stutchburyi	South and Southwest coast of Wellington / NZ	(0.006-0.008)		(Milne 2006)
Pipi				
Paphies australis	Lyttelton Harbour /NZ	0.03 (0.02 - 0.03)	0.11(0.07 - 0.13)	This study
Paphies subtriangulata	South and Southwest coast of Wellington / NZ	(0.010 - 0.010)		(Milne 2006)

Table 6.9: Comparison of lead concentrations in bivalve shellfish with existing literature

		Lead		
Species	Locations / description	Mean (range) µg g^{-1}		Reference
		Wet wt	Dry wt	
Mussel				
Perna canaliculus	Lyttelton Harbour / NZ	0.26 (0.15 - 0.41)	1.37 (0.78 - 2.14)	This study
Perna canaliculus	Bay of Islands / NZ	0.07 (0.03 - 0.10)		(Whyte et al. 2009)
Perna canaliculus	Wellington Harbour		14.1	(Kennedy 1986)
Perna canaliculus	All around NZ	1.8 (0.1 - 7.8)		(Nielsen & Nathan 1975)
Mytilus edulis	Wellington Harbour		(6.9 - 104.6)	(Kennedy 1986)
Mytilus galloprovincialis	South and Southwest coast of Wellington / NZ	(0.22 - 1.48)		(Milne 2006)
Cockle				
Austrovenus stutchburyi	Lyttelton Harbour /NZ	0.08 (0.03 - 0.13)	0.46 (0.16 - 0.72)	This study
Austrovenus stutchburyi	South and Southwest coast of Wellington / NZ	(0.06 - 0.14)		(Milne 2006)
Austrovenus stutchburyi	Avon-Heathcote Estuary / NZ		(0.3 - 1.37)	(Marsden et al. 2014)
Austrovenus stutchburyi	All around NZ	1.8		(Nielsen & Nathan 1975)
Pipi				
Paphies australis	Lyttelton Harbour /NZ	0.53 (0.05 - 0.24)	0.83 (0.25 - 0.13)	This study
Paphies australis	All around NZ	0.4		(Nielsen & Nathan 1975)
Paphies subtriangulata	South and Southwest coast of Wellington / NZ	(0.13 - 0.25)		(Milne 2006)

Table 6.10: Comparison of copper concentrations in bivalve shellfish with existing literature

Copper				
Species	Locations / description	Mean (range) µg g^{-1}		Reference
		Wet wt	Dry wt	
Mussel				
Perna canaliculus	Lyttelton Harbour /NZ	0.68 (0.71 - 0.98)	4.51 (3.73 - 4.72)	This study
Perna canaliculus	All around NZ	1.8 (0.2 - 28.0)		(Nielsen & Nathan 1975)
Perna canaliculus	Wellington Harbour		8.6	(Kennedy 1986)
Mytilus galloprovincialis	South and Southwest coast of Wellington / NZ	(0.55 - 1.34)		(Milne 2006)
Mytilus edulis	Wellington Harbour		(9.0 - 14.2)	(Kennedy 1986)
Cockle				
Austrovenus stutchburyi	Lyttelton Harbour /NZ	1.04 (0.88 - 1.22)	5.85 (4.93 - 7.11)	This study
Austrovenus stutchburyi	Otago Harbour and peninsula		(3 - 60)	(Peake et al. 2006)
Austrovenus stutchburyi	South and Southwest coast of Wellington / NZ	(0.86 - 1.35)		(Milne 2006)
Austrovenus stutchburyi	Avon-Heathcote Estuary / NZ		(10.1 - 21.9)	(Marsden et al. 2014)
Pipi				
Paphies australis	Lyttelton Harbour /NZ	1.25 (0.87 - 1.78)	6.01 (4.09 - 8.38)	This study
Paphies australis	All around NZ	1.0 (0.7 - 1.3)		(Nielsen & Nathan 1975)
Paphies subtriangulata	South and Southwest coast of Wellington / NZ	(1.37 - 1.47)		(Milne 2006)

Table 6.11: Comparison of iron concentrations in bivalve shellfish with existing literature

		Iron		
Species	Locations / description	Mean (range) µg g^{-1}		Reference
		Wet wt	Dry wt	
Mussel				
Perna canaliculus	Lyttelton Harbour /NZ	169 (150 - 212)	882 (783 - 1105)	This study
Perna canaliculus	Wellington Harbour		411	(Kennedy 1986)
Perna canaliculus	All around NZ	(26 - 280)		(Nielsen & Nathan 1975)
Mytilus edulis	Wellington Harbour		(235 - 457)	(Kennedy 1986)
Cockle				
Austrovenus stutchburyi	Lyttelton Harbour /NZ	76.78 (40.73 - 118)	433 (226 - 658)	This study
Austrovenus stutchburyi	Avon-Heathcote Estuary / NZ		(108 - 426)	(Marsden et al. 2014)
Austrovenus stutchburyi	Bay of Islands / NZ	31		(Nielsen & Nathan 1975)
Pipi				
Paphies australis	Lyttelton Harbour /NZ	72.37 (67.51 - 76.06)	349 (321 - 363)	This study
Paphies australis	All around NZ	(21 - 24)		(Nielsen & Nathan 1975)

Table 6.12: Comparison of zinc concentrations in bivalve shellfish with existing literature

		Zinc		
Species	Locations / description	Mean (range) µg g^{-1}		Reference
		Wet wt	Dry wt	
Mussel				
Perna canaliculus	Lyttelton Harbour /NZ	17.34 (14.67 - 21.82)	90.62 (77.02 - 113.50)	This study
Perna canaliculus	All around NZ	21 (0.5 - 28.0)		(Nielsen & Nathan 1975)
Perna canaliculus	Wellington Harbour		66.8	(Kennedy 1986)
Mytilus galloprovincialis	South and Southwest coast of Wellington / NZ	(28.0 - 49.8)		(Milne 2006)
Mytilus edulis	Wellington Harbour		(220 - 497)	(Kennedy 1986)
Cockle				
Austrovenus stutchburyi	Lyttelton Harbour /NZ	11.49 (10.96 - 12.62)	63.55 (60.68 - 69.22)	This study
Austrovenus stutchburyi	Otago Harbour and Peninsula		(40 - 118)	(Peake et al. 2006)
Austrovenus stutchburyi	South and Southwest coast of Wellington / NZ	(9.0 - 11.6)		(Milne 2006)
Austrovenus stutchburyi	Avon-Heathcote Estuary / NZ		(47.3 66.8)	(Marsden et al. 2014)
Austrovenus stutchburyi	All around NZ	10		(Nielsen & Nathan 1975)
Pipi				
Paphies australis	Lyttelton Harbour / NZ	12.32 (11.35 - 13.65)	58.50 (54.16 - 63.70)	This study
Paphies australis	All around NZ	13		(Nielsen & Nathan 1975)
Paphies subtriangulata	South and Southwest coast of Wellington / NZ	(8.8 - 10.5)		(Milne 2006)

Trace element concentrations in filter-feeding bivalves often reflect the contamination levels in the surrounding environment (Chandurvelan et al. 2015; Rainbow 1995). This has also been demonstrated in this study for arsenic, cadmium, and lead, which varied according to environmental contamination levels. For example, lead concentrations in green-lipped mussels from SB contained significantly higher concentrations than green-lipped mussels from other sites, and green-lipped mussels from the PG site contained significantly higher amounts of cadmium than most of the other sites. Similar trends were also reported by Kennedy (1986) in green-lipped mussels and blue mussels from different sites of Wellington Harbour.

The content of trace elements in burrowing bivalve species also reflects the concentrations measured in the sediments. For example, the highest content of lead was measured at the SB site while the highest concentrations of cadmium were measured in the PG site sediments. In general, this was reflected in bioaccumulation levels in cockles and pipi.

In addition to environmental influences, such as geochemical effects on bioavailability, levels of trace elements in shellfish also depend on the type of trace element, exposure route, and species-specific characteristics (Luoma & Rainbow 2005). Green-lipped mussels, cockles and pipi from the same sampling site contained significantly different amounts of some trace elements, indicating differences in trace element handling strategies, detoxification or excretion capacities. For instance, arsenic concentrations in cockles were predominantly higher than the green-lipped mussel or pipi from the same sites (at RP and PL), while the lead concentrations in green-lipped mussels were significantly higher than the other bivalve species investigated. This is supported by a study on different species of mussels from Wellington Harbour where the lead concentrations differed between two species collected from the same site (Kennedy 1986).

Research has also illustrated that trace element uptake routes can differ between species and for different trace elements (Pan & Wang 2009). Some species can accumulate certain types of trace elements to a greater degree than other species. For example, the green-lipped mussels of the current study contained significantly higher levels of mercury compared to the other two shellfish species. These observations suggest that the suspension feeding green-lipped mussels may become

enriched in mercury as it occupies a higher trophic level than the other two species of shellfish (Blackmore & Wang 2004; Reinfelder et al. 1998). During the filter feeding process, green-lipped mussels can collect zooplankton and phytoplankton that might have bioaccumulated mercury. The other two shellfish species (cockles and pipi) feed mainly on the sediment-bound materials and nutrients in the porewater (Marsden et al. 2014).

Copper and zinc showed a fairly similar range of concentrations among the bivalve species of this study, values that were also consistent with those reported previously for similar species (Tables 6.10 - 6.12). This may reflect the essentiality of these elements. As essential elements, copper and zinc are regulated within a certain optimal range, unlike toxic trace elements (cadmium, mercury or lead) (Blackmore & Wang 2004).

Both essential and non-essential elements can cause toxicity in bivalves when a certain threshold level is exceeded. The toxicity of trace elements in bivalves is generally determined by the species-specific partitioning of accumulated trace elements between metabolically active and metabolically less available detoxified forms (Luoma & Rainbow 2005; Rainbow 2002). When the uptake rate becomes greater than the detoxification rate or the excretion rate, balance will be disrupted (Luoma & Rainbow 2005; Pan & Wang 2009; Wang 2002). Hence, the metabolically available fraction of the trace elements will be increased, resulting in the appearance of toxic effects in the animal (Luoma & Rainbow 2005).

6.5 CONCLUSIONS

Iron, followed by zinc and then arsenic, were the most abundant trace elements in the three shellfish species investigated in the current study. Cadmium and mercury were found at very low concentrations in the three species of shellfish. The highest mean concentrations of arsenic, copper, iron and zinc were measured in the PL site sediments, while the PG site displayed the highest concentrations of cadmium, and the SB site contained the highest mean concentrations of lead. There were no significant correlations between shellfish lead, iron and zinc and the associated

sediments, although tissue cadmium (positive correlation) and copper (negative correlation) were correlated with sediment levels. This indicates that the concentrations in the sediment generally do not match the trace element levels in shellfish. The higher MPI values measured in the shellfish and sediments from the SB site were likely related to the presence of a sewage outfall in Governors Bay. The mean BSAF values obtained for the three species of this study suggests that, in general, green-lipped mussels have a greater capacity for trace element bioaccumulation than cockles or pipi, with the exception of arsenic and copper for cockles (Table 6.2). The mean BSAF values also indicate that cadmium has the highest efficiency for bioaccumulation from sediments, followed by arsenic and zinc.

None of the shellfish species in any site exceeded the maximum allowable value for inorganic arsenic, cadmium, mercury and lead in FSANZ or EC regulations. All the investigated trace element concentrations in the sediments of shellfish sites were well below the ANZECC ISQG-low trigger values recommended for contaminants in marine sediments. The PTWI values for inorganic arsenic, cadmium, mercury and lead were not exceeded at rates of fish and shellfish consumption by adults of the general population of New Zealand. Hence there is limited risk associated with consumption of fish and shellfish harvested from Lyttelton Harbour. However, adults can exceed the PTWI value for cadmium at high shellfish consumption rates (2849 g/week). This could be of concern for New Zealanders that rely heavily on seafood.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

7.1 OVERVIEW

This research was carried out to investigate whether trace elements were leaching from two sea-fill sites - Thilafushi Island in the Maldives and a sea-fill site in Lyttelton Harbour, New Zealand. The specific objectives were to determine if trace elements had accumulated in aquatic food chains due to the sea-fill activities, and to carry out risk assessment for consumption of seafood harvested from the vicinity of the sea-fills. To achieve the objectives of this research, multifaceted studies were designed and conducted at the two sea-fill sites. The following investigations were performed as separate individual components of this research.

1- Seawater and marine sediments were collected at selected distances along three transect lines from the two sea-fill sites (one transect line at a reference

site), and were analysed for trace elements.

2- Biota samples were collected from the vicinity of each sampling site to determine the concentrations of trace elements at different trophic levels in the food chain. The measured trace element concentrations were used to carry out a risk assessment for consumption of seafood harvested at the sea-fill sites.

3- A baseline study of trace element concentrations in shellfish was carried out for the wider Lyttelton Harbour, and a risk assessment for consumption of wild shellfish harvested in Lyttelton Harbour was completed.

This chapter presents summaries of all the research findings from the previous chapters with the main conclusions and recommendations for future work.

7.2 TRACE ELEMENTS IN THILAFUSHI ISLAND SEA-FILL, MALDIVES

7.2.1 ENVIRONMENTAL CHARACTERISATION

Overall, the seawater results indicated that concentrations of all trace elements were significantly higher at the sea-fill site (Thilafushi Island) than at the reference site (Huruelhi Island). For the sediments, all trace elements were significantly higher at the sea-fill site with the exception of arsenic and cadmium. These combined results indicate that the sea-fill of Thilafushi Island could be a source of trace elements to the surrounding marine environment with a noticeable decrease in element concentrations with distance along the T1 and T3 transects. The concentrations of trace elements were generally comparable to previously reported levels for seawater and sediments of sea-fill or coastal landfill sites elsewhere.

Seawater samples from Thilafushi Island exceeded the ANZECC trigger value for the protection of 80% of marine species for copper. Similarly, copper and zinc concentrations in marine sediments of the Thilafushi sea-fill exceeded the ANZECC guideline value of ISQG-low, and ISQG-high, respectively, implying that copper and zinc concentrations were high enough to have ecological effects at the sea-fill site

(Section 3.4.1).

7.2.2 TRACE ELEMENTS IN BIOTA

Marine biota collected at the sea-fill site contained significantly higher concentrations of copper, iron, mercury, lead and zinc compared to the reference site. Conversely, arsenic and cadmium were higher for the reference site. The cadmium result could be explained by competition for uptake between cadmium and the elevated levels of zinc at the sea-fill site. The trace element concentrations measured in the marine food chain species were generally similar to comparable species investigated elsewhere. Red mullet bioaccumulated significantly higher concentrations of arsenic, cadmium, copper, iron, lead and zinc than parrotfish, demonstrating that accumulation can differ between species under the same environmental exposure scenarios, although variations in diet can also play a role. Generally, the essential trace elements were measured at higher concentrations than non-essential trace elements.

Overall trace element concentrations were significantly lower in the fish muscle tissue than in liver, kidney and gonad. There were no significant differences in accumulated copper and iron (essential elements) between red mullet or parrotfish between the two sites, implying that these elements are regulated within these species at certain concentration ranges, even when exposed to higher environmental concentrations. However, despite zinc being an essential element it was found at significantly higher concentrations in parrotfish from the reference site compared to the sea-fill site. Zinc is known to play an important role in reproduction, and increases during the spawning period (Banks et al. 1999; Olsson et al. 1987). This study also found that zinc concentrations in gonad (reproductive organ of fish) were significantly higher than other soft tissues within the same fish, again likely related to the role of zinc in reproduction. Mercury was found at higher concentrations in the organisms at higher trophic levels relative to organisms lower down the food chain.

7.2.3 RISK ASSESSMENT FOR CONSUMPTION OF SEAFOOD HARVESTED AT THILAFUSHI SEA-FILL

Lead concentrations in all tissues of red mullet from the sea-fill site, and in the gonads and kidneys of parrotfish from the sea-fill site, exceeded the FSANZ

maximum allowable levels (ML) for fish (Section 3.4.3). Cadmium concentrations measured in the liver, kidney and gonad of red mullet of the Thilafushi sea-fill exceeded the EC ML value for fish (Section 3.4.3). Although zinc is an essential element, the concentrations measured in the shellfish from both the sampling sites exceeded the ML value for molluscs, suggesting that this species of shellfish (penguin wing oyster) can accumulate high concentrations of zinc. Utilising the accumulation values for fish muscle, it was shown that lead and arsenic intakes can exceed the respective provisional tolerable weekly intake (PTWI) values for all weight groups of human consumers (i.e. toddlers, children, adult males and females) (Section 3.4.3). These intakes were further increased when shellfish and fish organs were incorporated into the diet of Maldivians. Although only lead exceeded the ML values for fish, the high fish consumption rates in the Maldives can result in the PTWI for inorganic arsenic, methylmercury and lead for all weight groups being exceeded. The PTWI value of cadmium was only exceeded by toddlers when fish organs were incorporated in the diet with the fish muscle; while children can exceed the PTWI value for cadmium when shellfish is included with fish muscle and fish organs.

The trace element concentrations in seawater, marine sediments and biota of Thilafushi Island exceeded regulatory limits for those matrices. Furthermore, the results also showed that although the concentrations of some trace elements, such as arsenic and mercury, in seafood meet the existing ML levels, people can still exceed the PTWI values due to the high rates of fish consumption in the Maldives. Overall the dietary modelling showed that the sea-fill can increase dietary exposure to mercury and lead to levels above PTWIs. As there are no existing data of trace element concentrations of any biota or any food in the Maldives, the results of this study will serve as a baseline for future trace element studies in the Maldives, especially in terms of seafood for human risk assessment purposes.

7.3 TRACE ELEMENTS AT THE SEA-FILL OF LYTTELTON HARBOUR

7.3.1 MONITORING OF TRACE ELEMENTS AT THE SEA-FILL

Overall, seawater and marine sediment data indicated that trace elements at the LH sea-fill site were significantly higher than at the reference site, with the exception of arsenic in sediment which was not different between the two sites. Concentrations of cadmium, lead and zinc in seawater samples were significantly higher closer to the silt curtain and decreased with distance from the sea-fill. In the sediments, the arsenic, cadmium and lead concentrations decreased with distance from the sea-fill. This is strong evidence that the sea-fill is leaching trace elements to the surrounding aquatic environment. Sediment arsenic and iron concentrations increased over time at the sea-fill site over the sampling period, while copper decreased. The levels of trace elements measured in seawater and sediments in the vicinity of the sea-fill were generally comparable to previously reported levels for sea-fill or coastal landfill sites elsewhere.

The results from biota monitoring generally supported the data collected for sediment and seawater. Green-lipped mussels from the sea-fill site showed higher levels of tissue burdens than animals from the reference site, with the sole exception of cadmium. However, data also indicated that seasonal variations, possibly associated with factors such as spawning, played a role. Placing these accumulation data in a regulatory context, it was shown that copper in seawater and lead in sediment from the sea-fill site were at levels where biological impacts could be expected. It is, however, unlikely that effects would be observed in human consumers of seafood harvested from this site.

7.3.2 TRACE ELEMENTS IN A COASTAL FOOD CHAIN, AND TROPHIC TRANSFER POTENTIAL

This study was the first to examine the passage of trace elements through a food chain in Lyttelton Harbour, and to show how this potentially links environmental trace elements with human consumption. The data presented here showed that iron and lead actually diluted in concentration in higher trophic levels (Section 5.3.3). This

is likely explained by mass dilution, and/or the greater importance of alternative (non-dietary) exposure routes in lower trophic feeders. Mercury was the only element found to appreciably biomagnify through the food chain (Section 5.3.3). This finding is consistent with the high bioavailability of methylmercury. Together these data show that the diet is a key pathway of exposure in food chains, but dissolved trace elements and sediment exposures will also play a role in trace element body burdens.

The trace elements likely to be of greatest relevance in Lyttelton Harbour are cadmium and mercury, which both exceeded regulatory limits in fish liver and kidney (Section 5.3.4). This indicates these elements may cause health risks from regular consumption of these organs. It was shown, however, that Lyttelton Harbour remains a relatively clean site. Trace element concentrations found in the food chain species of the LH sea-fill site were generally lower than in other regions of New Zealand (Brooks & Rumsey 1974; Kennedy 1986; Love et al. 2003; Nielsen & Nathan 1975; Whyte et al. 2009).

7.3.3 BASELINE STUDY OF SHELLFISH AND RISK ASSESSMENT FOR CONSUMPTION OF SEAFOOD HARVESTED IN LYTTELTON HARBOUR

The next phase of the work focussed on a broader examination of trace element profiles around Lyttelton Harbour, by examining trace element burdens, and their relationships to sediment concentrations, in three shellfish species. Different elements were elevated at different locations, indicating distinct point sources for trace element contaminants around the harbour. However, no sediment sample exceeded the ANZECC ISQG-low/high values for any trace element. Of the shellfish species tested, green-lipped mussels accumulated the highest levels of trace elements, with the exception of arsenic in cockles and copper in pipi. Sandy Beach (SB) was shown to be the most contaminated site by applying an MPI approach with biota trace elements (Section 6.3.2; Table 6.2), likely the consequence of a sewerage outfall at nearby Governor’s Bay. Port Levy (PL) was the highest scoring site if sediment trace element concentrations were used for MPI calculations. Biota sediment accumulation factors showed that cadmium had the highest efficiency for bioaccumulation from sediments, followed by arsenic and zinc.

No shellfish species at any site in the wider Lyttelton Harbour exceeded the

maximum allowable values for inorganic arsenic, cadmium, mercury and lead. The PTWI values for inorganic arsenic, cadmium, mercury and lead were not exceeded at rates of fish and shellfish consumption by adults of the general population of New Zealand. However, adults can exceed the PTWI value for cadmium at high shellfish consumption rates (2849 g/week) (Section 6.3.2.11; Table 6.4). This could be of concern for populations that rely heavily on seafood.

7.4 SYNTHESIS OF KEY FINDINGS

The key findings from this thesis include:

- Sea-fill sites are a potential source of trace elements to marine ecosystems.
- The body burdens of marine organisms reflect environmental concentrations of trace elements.
- Body burden of trace elements generally depends on the species, the essentiality of trace element, and the metal handling strategies adopted by different species.
- Different trace elements behave differently in different organisms in the food chain, and their toxicity can vary depending on the chemical speciation they present in the biota.
- High consumers of seafood can exceed PTWIs despite trace element concentrations being below maximum allowable limits.

7.5 RECOMMENDATIONS FOR FURTHER WORK

7.5.1 MALDIVES STUDY

This was the first study to investigate trace element (arsenic, cadmium, copper, iron, mercury, lead and zinc) concentrations in biota from the Maldives. The

samples collected from the Thilafushi sea-fill site of Maldives had levels of copper in seawater and zinc in sediment that exceeded water quality guidelines for the protection of marine species (Section 3.4.1). These data are an initial insight into potential sources and magnitudes of contamination in this island nation, and will provide a baseline understanding of trace element concentrations that can be utilised for future monitoring purposes. Ultimately it is hoped that these results will lead to better protection of marine species, and a greater understanding of the risks to human consumers of these organisms. To achieve this, more thorough surveys are required. These should employ the multi-matrix approach validated in the current study, but encompass a greater area and a wider range of biota. This latter point is particularly important given the significant species-differences highlighted throughout the current thesis. Of specific value would be studies examining highly consumed fish species such as tuna. Tuna is well known to contain high levels of trace elements, especially mercury, due to its higher trophic position (Olmedo et al. 2013; Storelli 2008; Storelli et al. 2012).

Importantly, it is clear that there is discordance between the maximum allowable trace element levels in biota and human regulatory limits. This is largely a consequence of the very high fish consumption rates of Maldivians, and is a factor that should be accounted for in setting human risk assessment advisories. Another key factor is the consumption of fish organs, shown in this and other studies, to contain elevated trace element levels, and which are considered a delicacy in this country. It is also recommended that a total diet survey be performed in the Maldives, as no such data exists. This would be critical for determining dietary exposure to trace elements and other environmental contaminants (see below) that find their way into the human food chain.

It is worth highlighting that trace elements are not the only contaminants that could potentially be leaching from the Thilafushi Island sea-fill and entering marine food chains. Assessment of toxic organic compounds is strongly recommended. Organo-tin compounds from anti-fouling paints are also likely to be present at the Thilafushi sea-fill site, as the inner lagoon of Thilafushi Island is a sheltered location for boats to anchor for various purposes including repair and maintenance work. Furthermore, although toxicants such as polychlorinated biphenyls (PCBs),

polyaromatic hydrocarbons (PAHs), and brominated flame retardants were not considered in the current thesis, they are likely to be present in sea-fill sites, and are likely to accumulate in food chains, and pose risks to seafood consumers (Cooney & Wuertz 1989; Farrington et al. 1983; Jones 2010; Jones 2011; Kjeldsen et al. 2002; Storelli 2008).

7.5.2 NEW ZEALAND STUDY

Copper concentrations in the seawater and lead concentrations in the marine sediment of the LH sea-fill site exceeded the recommended limits for the protection of marine species (Section 4.4.1.4). Therefore, longer-term monitoring work of trace element concentrations in different matrices at the sea-fill site is recommended. More species of biota from different trophic levels, covering all the components of the diet for each key species investigated in the food chain of LH sea-fill site should be analysed to confirm trophic transfer potential and biomagnification of individual trace elements.

The target contaminants should be extended to include organo-tin compounds and nickel. Lyttelton Harbour is a busy port with extensive shipping activities, and therefore historical use of organo-tin compounds as anti-fouling paints is likely to have left an impact in the environment. Nickel is an important trace element associated with many of the likely sea-fill constituents, and thus could also be a contaminant of concern (Jones 2010; Kjeldsen et al. 2002). Both organo-tins and nickel have been shown to have deleterious effects on marine biota (Cooney & Wuertz 1989; Madoni 2000; Münzinger 1990).

This study highlighted higher bioavailablility for cadmium at a site considered to be a suitable reference site (Section 4.4.2) for LH. An investigation should be carried out to find the possible sources of cadmium and the reasons for higher bioavailability of cadmium in Pigeon Bay compared to Lyttelton Harbour.

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APPENDICES

APPENDIX - A

A1: Sample collection site data (GPS co-ordinates, depth of sediment collection, pH of seawater) for studies conducted in Maldives

Site (transect line-T1) - sea-fill site	**1A (2m)**	**1B (20 m)**	**1C (80 m)**	**1D (160 m)**	**1E (400 m)**
Depth	3.5 m	6.4 m	7 m	7.2 m	6.3 m
pH of seawater	7.5	7.5	7.6	7.7	7.7
GPS coordinates	N 4° 10' 56.5464"	N 4° 10' 56.7696"	N 4° 10' 57.3492"	N 4° 10' 58.2234"	N 4° 11' 1.269"
	E 73° 26' 53.2566"	E 73° 26' 52.6056"	E 73° 26' 50.8122"	E 73° 26' 48.3174"	E 73° 26' 41.085"
Site (transect line-T2) - sea-fill site	**2A (2 m)**	**2B (20 m)**	**2C (80 m)**	**2D (160 m)**	**2E (400 m)**
Depth	0.7 m	0.8 m	0.9 m	1 m	1 m
pH of seawater	8.1	8	7.9	7.8	8.2
GPS coordinates	N 4° 10' 58.7526"	N 4° 10' 58.7706"	N 4° 10' 58.9794"	N 4° 10' 59.1378"	N 4° 10' 59.8872"
	E 73° 25' 35.544"	E 73° 25' 34.8924"	E 73° 25' 32.97"	E 73° 25' 30.3564"	E 73° 25' 22.5948"
Site (transect line-T3) - sea-fill site	**3A (2 m)**	**3B (20 m)**	**3C (80 m)**	**3D (160 m)**	**3E (400 m)**
Depth	0.45 m	0.78 m	1.8 m	2.9 m	10.5 m
pH of seawater	8	8	8	8.1	8.1
GPS coordinates	N 4° 10' 44.4354"	N 4° 10' 44.0544"	N 4° 10' 43.0782"	N 4° 10' 41.757"	N 4° 10' 37.7904"
	E 73° 26' 52.6086"	E 73° 26' 53.1456"	E 73° 26' 54.7944"	E 73° 26' 57.0588"	E 73° 27' 3.744"
Site (transect line-T4) - reference site	**4A (2 m)**	**4B (20 m)**	**4C (80 m)**	**4D (160 m)**	**4E (400 m)**
Depth	0.36 m	0.75 m	1.8 m	2.5 m	11.1 m
pH of seawater	8.2	8.2	8.2	8.2	8.2
GPS coordinates	N 3° 32' 42.4644"	N 3° 32' 43.08"	N 3° 32' 44.9772"	N 3° 32' 47.4972"	N 3° 32' 54.8298"
	E 72° 43' 10.9848"	E 72° 43' 11.1714"	E 72° 43' 11.766"	E 72° 43' 12.5682"	E 72° 43' 14.9982"

Sample site	**GPS coordinates of biota samples**				
	Fish	Crab	Shellfish	Worm	Algae
Thilafushi Island	N 4° 11' 17.5272"	N 4° 11' 4.2858"	N 4° 11' 26.0838"	N 4° 11' 8.628"	N 4° 11' 2.5656"
	E 73° 25' 42.636"	E 73° 24' 42.0762"	E 73° 25' 2.7078"	E 73° 25' 25.1472"	E 73° 26' 5.4888"
Huruelhi Island	N 3° 32' 50.8554"	N 3° 32' 24.831"	N 3° 32' 30.6816"	N 3° 32' 38.2308"	N 3° 32' 37.917"
	E 72° 43' 8.7414"	E 72° 43' 5.0514"	E 72° 43' 1.7832"	E 72° 43' 16.5108"	E 72° 43' 8.5398"

A2: Overview of sampling species from Thilafushi Island and Huruelhi Island of Maldives

Scientific name	Common name	Sample site	Length (mm)		Whole body weight (g), (shell included where applicable)		Replicates (n)
Fish			Mean	Range	Mean	Range	
Parupeneus indicus	Red mullet or Indian goatfish	Thilafushi	282.8 ± 21.6	250 - 315	419.2 ± 90.8	272 - 559	12
Parupeneus indicus	Red mullet or Indian goatfish	Huruelhi	201.2 ± 39.4	160 - 280	157.8 ± 96.5	72 - 352	12
Scarus ventula	Queen parrotfish	Thilafushi	317.8 ± 55.5	200 - 395	686.5 ± 254.5	357 - 1171	12
Scarus ventula	Queen parrotfish	Huruelhi	288.9 ± 36.8	235 - 370	468.8 ± 120.1	277 - 689	12
Shellfish							
Pteria penguin	Penguin wing oyster	Thilafushi	206.2 ± 42.1	165 - 265	367.8 ± 188.7	147 - 815	12
Pteria penguin	Penguin wing oyster	Huruelhi	203.5 ± 29.8	140 - 231	317.8 ± 147.3	112 - 573	12
Marine worm							
Sipunculus indicus	Peanut worm	Thilafushi	NA	NA	21.9 ± 5.8	13.5 - 31.2	12
Sipunculus indicus	Peanut worm	Huruelhi	NA	NA	10.3 ± 4.2	3.96 - 15.8	12
Green algae							
	Green algae	Thilafushi	NA	NA	4.5 ± 1.5	2.1 - 6.7	12
	Green algae	Huruelhi	NA	NA	2.2 ± 0.6	1.4 - 3.6	12

NA- not applicable

A3: Analytical performance results

SEAWATER

Percentage recoveries of cadmium, copper, iron, lead and zinc in certified reference seawater and trace elements spiked Milli-Q water, real seawater and artificial seawater ranged from 83 to 122% (Table A3.1). Quality assurance (QA) water samples were extracted in duplicate.

Table A3.1: Mean percentage recoveries of trace elements in QA water samples

Analyte	% recovery of trace elements in certified reference seawater and trace element spiked water samples							
	CRM	Milli-Q (n=2)		Real seawater (n=2)			Artificial seawater (n=2)	
	NASS-6 (n=2)	1 ppb	10 ppb	1 ppb	5 ppb	10 ppb	1 ppb	10 ppb
Cadmium	108.3	104.6	99.4	98.3	102.4	100.0	101.0	97.0
Copper	96.1	98.5	95.7	93.0	100.8	89.0	97.6	99.0
Iron	<LOD	89.7	85.3	82.6	96.9	91.0	89.1	92.7
Lead	122.0	95.3	100.2	90.6	89.9	82.8	92.6	88.7
Yttrium	89.6	90.3	94.6	90.9	88.9	87.5	98.2	91.9
Zinc	116.5	89.0	98.0	92.2	99.4	89.7	97.6	95.8

CRM (NASS-6) -certified reference materials (seawater); <LOD - below limit of detection.

The percentage recovery for iron ranged from 83 to 97% for the trace element-spiked samples. The possible reasons for this relatively low recovery were previously discussed in Chapter 2 (Section 2.2.4). Yttrium was used as an internal standard in this work and had a recovery range from 89 to 98%. Every 10^{th} sample of environmental seawater was extracted in duplicate for checking the accuracy of the extraction method. The percentage differences in the duplicate extractions were less than 12% for all elements, but 15.7% for zinc.

MARINE SEDIMENTS

The percentage recoveries for the marine sediment standard reference materials (SRM-2702-NIST) digested and analysed in duplicate along with the environmental sediment samples ranged from 92 to 115%. The mean percentage recoveries of arsenic, cadmium, copper, iron, mercury, lead and zinc in the standard reference sediment were 105.8, 114.7, 96.8, 93.6, 91.5, 98.5 and 96.8 respectively.

The percentage differences of the 10th sample duplicate digests were within 5% for all analytes except arsenic (11%). Every 10th sample was analysed in duplicate and every 20th sample was analysed with low level trace element spike for checking analytical instrument performance (ICP-MS). These duplicate sample differences were less than 5.5% for all elements. The percentage recoveries of the spiked elements were between 91-113%.

BIOTA

Standard reference material of mussel tissue (SRM-2976, EVISA) and fish protein (DORM-3) were digested in duplicate along with the environmental fish muscle, crab muscle and shellfish samples. Standard reference bovine liver (SRM 1557c, NIST), mussel tissue (SRM-2976, EVISA) and fish protein (DORM-3) were digested along with the fish organs (liver, kidney and gonad) and marine worm samples. Tomato leaves (SRM 1573a, EVISA) were digested in duplicate along with the green algae samples. The mean percentage recoveries of the standard reference materials are provided in Table A3.2.

Table A3.2: Mean percentage recoveries of standard reference materials

Analytes	Mean % recoveries of trace elements in standard reference materials			
	Mussel (n = 4)	DORM-3 (n = 4)	Bovine liver (n = 4)	Tomato leaves (n = 2)
Arsenic	119.0 ± 4.3	97.5 ± 2.8	< LOQ	< LOQ
Cadmium	106.4 ± 2.1	103.7 ± 1.6	103.2 ± 3.6	105.2± 7.2
Copper	94.7 ± 4.2	91.0 ± 2.4	90.7 ± 4.1	104.3 ± 3.7
Iron	106.3 ± 5.5	95.9± 2.9	100.9 ±8.5	98.5 ± 10.6
Mercury	98.7 ± 13.5	91.8 ± 8.4	< LOQ	< LOQ
Lead	96.8 ± 2.4	34.9 ± 4.2	88.7 ± 5.7	NP*
Zinc	106.0 ± 2.9	103.0 ± 9.4	96.0 ± 4.1	104.0 ±12.3

***NP**-data not provided, **LOQ**- limit of quantification

The percentage recoveries of all elements in the standard reference mussel and fish protein (DORM-3) were over 90% with the exception of lead for DORM-3. The percentage recovery of lead in DORM-3 was 34.9%, which was comparable with the results obtained in the method validation work described in Chapter 2.

The percentage differences of the 10th sample duplicate digests were less than 10% for all the biota species. The percentage differences of the 10th sample duplicate analyses (instrument check- ICP-MS) were within 10% for all elements for all biota

species with the exception of fish muscle iron (13%), and mercury in kidney and gonad samples (11%).

The percentage recoveries of the spiked elements ranged from 93 to 116% for fish muscle, from 85 to 98% for shellfish, from 92 to 103% for marine worms (with the exception of arsenic; 131%), and from 86 to 126% for green algae. It is worth highlighting the percentage recoveries for the spiked elements in the green algae samples were 126%, 97% and 86% for arsenic, lead and mercury respectively. The percentage recoveries of the spiked elements in all the fish organs ranged from 91 to 118% for all elements except arsenic in kidney samples (135%).

A4: Trace element concentrations in marine sediment and seawater collected along the transect lines at the sea-fill and the reference site (Huruelhi)

Trace element concentrations in sediment (mg kg dry wt^{-1})						Site	Transect	Distance from Shoreline (m)	Trace element concentrations in seawater (µg L^{-1})				
Fe	Cu	Zn	As	Cd	Pb				Fe	Cu	Zn	Cd	Pb
13562.82	148.32	425.81	6.08	0.44	30.69	Thilafushi	T1	2	28.41	1.28	4.38	0.018	0.223
1545.68	31.29	68	3.94	0.24	26.21	Thilafushi	T1	20	28.89	1.46	4.05	0.018	0.200
2316	55.14	59.32	5.1	0.17	18.12	Thilafushi	T1	80	26.29	1.61	4.33	0.017	0.186
1789.95	44.67	69.62	4.76	0.21	16.6	Thilafushi	T1	160	24.01	1.27	3.84	0.017	0.167
741.2	14.06	19.66	3.63	0.08	5.96	Thilafushi	T1	400	25.21	1.31	4.38	0.017	0.165
32.31	0.14	0.23	1.83	0.06	0.23	Thilafushi	T2	2	11.75	0.31	1.18	0.019	0.035
26.16	0.14	0.47	1.61	0.05	0.24	Thilafushi	T2	20	10.64	0.22	0.98	0.015	0.026
22.56	0.15	0.41	1.49	0.04	0.24	Thilafushi	T2	80	9.97	0.17	0.88	0.014	0.023
35.31	0.14	0.3	1.82	0.04	0.26	Thilafushi	T2	160	9.95	0.17	1.14	0.014	0.060
29.15	0.16	0.4	2.51	0.05	0.2	Thilafushi	T2	400	10.67	0.2	0.97	0.015	0.027
712.96	49.12	13.66	1.77	0.06	4.62	Thilafushi	T3	2	31.44	16.37	5.26	0.019	0.848
401.17	11.38	7.08	2.03	0.07	1.82	Thilafushi	T3	20	16.27	2.1	2.09	0.016	0.505
616.04	11.15	8.13	2.28	0.17	2.62	Thilafushi	T3	80	17.1	1.37	1.94	0.016	0.376
93.39	0.6	1.37	2.75	0.07	0.56	Thilafushi	T3	160	10.61	0.26	2.84	0.012	0.049
61.25	0.56	1.01	2.14	0.07	0.45	Thilafushi	T3	400	9.77	0.18	1.38	0.012	0.031
25.29	0.15	0.21	1.45	0.05	0.41	Huruelhi	T4	2	10.52	0.16	1.02	0.013	0.019
30.35	0.19	0.25	1.63	0.07	0.42	Huruelhi	T4	20	9.75	0.14	0.86	0.013	0.020
28.88	0.2	0.22	1.75	0.07	0.39	Huruelhi	T4	80	10.3	0.15	0.84	0.012	0.020
28.38	0.21	0.26	2.37	0.08	0.45	Huruelhi	T4	160	10.55	0.15	1.02	0.014	0.016
27.24	0.2	0.35	1.9	0.07	0.26	Huruelhi	T4	400	10.76	0.15	0.94	0.011	0.018

A5: Pearson's correlation tests for seawater and marine sediments

Table A5.1: Pearson's correlations coefficients and p-values for significant relationships (bold) between trace element concentrations in seawater for both the sampling sites

Thilafushi	**Fe** Corr. coefficient	*P* value	**Cu** Corr. coefficient	*P* value	**Zn** Corr. coefficient	*P* value	**Cd** Corr. coefficient	*P* value	**Pb** Corr. coefficient	*P* value
Fe	1.0									
Cu	**0.554**	**0.032**	1.0							
Zn	**0.944**	**0.000**	**0.555**	**0.032**	1.0					
Cd	**0.784**	**0.001**	**0.498**	**0.059**	**0.642**	**0.010**	1.0			
Pb	**0.612**	**0.015**	**0.867**	**0.000**	**0.563**	**0.029**	**0.559**	**0.030**	1.0	
Huruelhi	**Fe** Corr. coefficient	*P* value	**Cu** Corr. coefficient	*P* value	**Zn** Corr. coefficient	*P* value	**Cd** Corr. coefficient	*P* value	**Pb** Corr. coefficient	*P* value
Fe	1.0									
Cu	0.705	0.183	1.0							
Zn	0.631	0.254	0.663	0.223	1.0					
Cd	-0.319	0.600	0.115	0.854	0.467	0.428	1.0			
Pb	-0.622	0.262	-0.211	0.733	-0.749	0.145	-0.230	0.709	1.0	

Table A5.2: Pearson's correlations coefficients and p-values for significant relationships (bold) between trace elements in sediments for both the sampling sites

Thilafushi	Fe		Cu		Zn		As		Cd		Pb	
	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value
Fe	1.000											
Cu	**0.942**	**0.000**	1.000									
Zn	**0.997**	**0.000**	**0.937**	**0.000**	1.000							
As	**0.746**	**0.001**	**0.800**	**0.000**	**0.757**	**0.001**	1.000					
Cd	**0.891**	**0.000**	**0.886**	**0.000**	**0.906**	**0.000**	**0.855**	**0.000**	1.000			
Pb	**0.761**	**0.001**	**0.836**	**0.000**	**0.786**	**0.001**	**0.896**	**0.000**	**0.915**	**0.000**	1.000	
Huruelhi	Fe		Cu		Zn		As		Cd		Pb	
	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value
Fe	1.000											
Cu	0.659	0.226	1.000									
Zn	0.021	0.974	0.481	0.412	1.000							
As	0.243	0.694	0.791	0.111	0.393	0.513	1.000					
Cd	0.702	0.186	**0.973**	**0.005**	0.404	0.500	0.844	0.072	1.000			
Pb	0.248	0.688	-0.130	0.835	-0.798	0.105	0.092	0.883	0.050	0.937	1.000	

Table A5.3: Pearson's correlations coefficients and *p*-values for significant relationships (bold) between trace elements in seawater and trace elements in sediments for both the sampling sites

Thilafushi		**Sediment**									
		Fe		Cu		Zn		Cd		Pb	
		Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value
Seawater	Fe	0.497	0.060								
	Cu			0.245	0.379						
	Zn					0.450	0.092				
	Cd							0.422	0.117		
	Pb									0.118	0.676
Huruelhi		**Sediment**									
		Fe		Cu		Zn		Cd		Pb	
		Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value	Corr. coefficient	*P* value
Seawater	Fe	-0.736	0.156								
	Cu			-0.603	0.282						
	Zn					0.040	0.949				
	Cd							-0.114	0.855		
	Pb									-0.057	0.928

A6: Trace element concentrations in fish tissues

Fish samples	Concentration (µg g wet wt^{-1}) of trace elements in fish and fish organs (mean ± SE)						
	Total As	Cd	Cu	Fe	Hg	Pb	Zn
Thilafushi Island fish							
Red mullet or Indian goatfish (*Parupeneus indicus*), n=12							
Muscle	8.27 ± 1.71	0.01 ± 0.01	0.20 ± 0.03	5.51 ± 0 .73	0.05 ± 0.02	1.06 ± 1.82	7.17 ± 1.24
Liver	14.91 ± 3.38	8.84 ± 3.44	12.02 ± 3.75	920 ± 197	0.05 ± 0.02	15.42 ± 40.26	40.15 ± 5.65
Kidney	13.31 ± 2.18	0.51 ± 0.26	1.59 ± 0.15	884 ± 316	0.08 ± 0.03	9.85 ± 27.21	1051 ± 540
Gonad	10.68 ± 3.55	0.26 ± 0.14	1.09 ± 0.11	53.33 ± 13.04	0.03 ± 0.02	0.92 ± 1.11	61.76 ± 44.27
Parrotfish (*Scarus ventula)*, n=12							
Muscle	0.65 ± 0.23	<LOQ	0.16 ± 0.04	2.16 ± 0.86	<LOQ	0.09 ± 1.4	3.25 ± 0.22
Liver	1.81 ± 0.33	4.57 ± 2.35	5.46 ± 2.73	157 ± 119	0.02 ± 0.01	0.30 ± 0.45	25.29 ± 8.43
Kidney	1.04 ± 0.27	2.70 ± 1.18	1.56 ± 0.42	193 ± 61.18	0.05 ± 0.01	0.86 ± 1.04	16.47 ± 3.50
Gonad	2.33 ± 1.60	0.25 ± 0.18	0.55 ± 0.26	11.70 ± 7.77	<LOQ	1.49 ± 2.96	15.26 ± 7.42
Huruelhi Island Fish							
Red mullet or Indian goatfish (*Parupeneus indicus*), n=12							
Muscle	12.93 ± 5.31	0.01 ± 0.01	0.13 ± 0.01	2.84 ± 0.43	0.03 ± 0.02	<LOQ	6.17 ± 1.30
Liver	16.44 ± 4.76	8.42 ± 3.68	13.48 ± 3.40	735 ± 183	0.05 ± 0.04	0.08 ± 0.04	39.90 ± 7.16
Kidney	16.84 ± 4.63	0.73 ± 0.43	1.58 ± 0.35	424 ± 208	0.08 ± 0.05	0.04 ± 0.03	566 ± 329
Gonad	17.14 ± 2.25	0.39 ± 0.36	1.70 ± 0.80	44.74 ± 13.72	<LOQ	0.01 ± 0.02	159 ± 34
Parrotfish (*Scarus ventula)*, n=12							
Muscle	0.71 ± 0.08	<LOQ	0.12 ± 0.01	1.51 ± 0.38	<LOQ	<LOQ	3.31 ± 0.43
Liver	2.19 ± 0.32	12.05 ± 8.24	7.26 ± 3.09	194 ± 107	0.03 ± 0.02	0.08 ± 0.07	31.09 ± 14.3
Kidney	1.16 ± 0.22	5.71 ± 2.07	1.24 ± 0.20	245 ± 61.67	0.07 ± 0.03	0.21 ± 0.13	18.99 ± 5.55
Gonad	2.37 ± 2.53	0.51 ± 0.43	0.56 ± 0.21	23.96 ± 18.17	<LOQ	0.02 ± 0.03	74.08 ± 62.87

A7: Trace element concentrations in shellfish, marine worm and green algae samples

Biota Samples	Concentration (µg g wet wt^{-1}) of trace elements in marine biota (mean ± SE)						
	Total As	Cd	Cu	Fe	Hg	Pb	Zn
Thilafushi Island biota							
Penguin Wing Oyster (*Pteria penguin*) , n = 12	13.83 ± 6.97	2.07 ± 0.01	0.79 ± 0.11	30.89 ± 9.30	0.03 ± 0.01	0.16 ± 0.11	415 ± 181
Peanut worm (*Sipunculus indicus*), n = 12	3.60 ± 0.77	0.36 ± 0.09	0.33 ± 0.06	64.31 ± 3.99	<LOQ	0.33 ± 0.08	9.25 ± 0.93
Green algae, n = 12	1.54 ± 0.37	0.02 ± 0.00	14.67 ± 1.72	243 ± 26.14	<LOQ	1.62 ± 0.27	9.20 ± 1.16
Huruelhi Island biota							
Penguin Wing Oyster (*Pteria penguin*) , n = 12	11.03 ± 2.99	1.53 ± 0.46	0.83 ± 0.16	14.21 ± 6.23	0.02 ± 0.01	0.06 ± 0.02	125 ± 55.17
Peanut worm (*Sipunculus indicus*), n = 12	13.48 ± 1.55	0.68 ± 0.10	0.43 ± 0.08	15.26 ± 2.01	<LOQ	0.04 ± 0.01	5.31 ± 1.15
Green algae, n = 12	2.17 ± 0.43	0.05 ± 0.01	0.73 ± 0.26	30.93 ± 5.00	<LOQ	0.09 ± 0.02	0.71 ± 0.41

APPENDIX - B

B1: GPS coordinates, distance from shoreline of seawater and sediment sites, and the depth of sediment samples collected at the transect lines of the LH sea-fill (Chapter 4)

Transect line-T1	**1A (2 m)**	**1B (20 m)**	**1C (80 m)**	**1D (160 m)**	**1E (400 m)**
Depth	8 m	8.6 m	8.6 m	8.6 m	12.7 m
GPS coordinates	S 43° 36.530'	S 43° 36.553'	S 43° 36.578'	S 43° 36.614'	N 43° 36.715'
	E 172° 44.026'	E 172° 44.029'	E 172° 44.054'	E172° 44.093'	E 172° 44.200'
Transect line-T2	**2A (2 m)**	**2B (20 m)**	**2C (80 m)**	**2D (160 m)**	**2E (400 m)**
Depth	7.7 m	7.8 m	7.8 m	7.3 m	12.6 m
GPS coordinates	S 43° 36.497'	S 43° 36.510'	S 43° 36.519'	S 43° 36.544'	S 43° 36.616'
	E 172° 44.117'	E 172° 44.130'	E 172° 44.168'	E 172° 44.220'	E 172° 44.379'
Transect line-T3	**3A (2 m)**	**3B (20 m)**	**3C (80 m)**	**3D (160 m)**	**3E (400 m)**
Depth	6.4 m	6.5 m	6.5 m	7.0 m	8.0 m
GPS coordinates	S 43° 36.349'	S 43° 36.347'	S 43° 36.346'	S 43° 36.327'	S 43° 36.295'
	E 172° 44.138'	E 172° 44.157'	E 172° 44.199'	E 172° 44.0255'	E 172° 44.427'
Transect line-T4	**4A (2m)**	**4B (20m)**	**4C (80m)**	**4D (160m)**	**4E (400m)**
Depth	4.2 m	4.2 m	4.4 m	4.5 m	5.1 m
GPS coordinates	S 43° 40.313'	S 43° 40.304'	S 43° 40. 289'	S 43° 40. 267'	S 43° 40.192'
	E 172° 53.304'	E 172° 53.311'	E 172° 53.322'	E 172° 53.336'	E 172° 53.377'
GPS coordinates and depths of seawater and sediment samples from sites around Lyttelton Harbour (Chapter 4, Figure 4.2)					
Site	**1**	**2**	**3**	**4**	**5**
Depth	1.3 m	1.6 m	2.1 m	3.1 m	3.5 m
GPS coordinates	S 43° 38.744'	S 43° 38.844'	S 43° 37.562'	S 43° 36.667'	S 43° 37.276'
	E 172° 40.424'	E 172° 42.036'	E 172° 39.532'	E 172° 40.614'	E 172° 40.453'
Site	**6**	**7**	**8**	**9**	**10**
Depth	5.5 m	5.0 m	7.5 m	8.0 m	4.2 m
GPS coordinates	S 43° 37.065'	S 43° 37.483'	S 43° 37.389'	S 43° 36.530'	S 43° 37.681'
	E 172° 42.001'	E 172° 43.045'	E 172° 44.035'	E 172° 44.026'	E 172° 44.904'
Site	**11**	**12**	**13**		
Depth	9.6 m	13.2 m	15.4 m		
GPS coordinates	S 43° 36.604'	S 43° 36.238'	S 43° 35.849'		
	E 172° 45.366'	E 172° 47.157'	E 172° 49.149'		

B2: Percentage recoveries of trace elements in quality control

Table B2.1: Mean percentage recoveries of trace elements in quality control water samples (Chapter 4)

Analyte	% recovery of trace elements in certified reference seawater and metal-spiked water samples							
	CRM	Milli-Q water (n = 12)		Natural seawater (n = 6)			Artificial seawater (n = 12)	
	NASS-6 (n = 6)	1 ppb	10 ppb	1 ppb	5 ppb	10 ppb	1 ppb	10 ppb
Cadmium	99.7	91.7	93.4	96.6	95.1	93.4	96.0	94.2
Copper	103.7	102.7	100.5	90.3	96.3	87.4	97.6	95.1
Iron	<LOQ	86.4	96.6	85.3	118.2	93.2	84.7	93.5
Lead	119.9	90.6	96.4	91.0	90.0	88.6	88.1	90.3
Yttrium	91.2	92.6	97.2	89.8	86.5	90.3	89.3	88.8
Zinc	96.6	90.9	92.7	91.2	107.1	95.3	98.8	96.1

Table B2.1 represents percentage recoveries of trace elements in the quality control samples for the seawater of Chapter 4. Percentage recoveries of cadmium, copper, iron, lead, yttrium and zinc in certified reference seawater (NASS-6), and trace element-spiked Milli-Q water, natural seawater and artificial seawater were within acceptable ranges (85-120%). The recovery for iron ranged from 85-118% for all the spiked samples. Yttrium was used as an internal standard in this work and had a recovery range from 89 to 97%. Every 10^{th} sample of seawater was extracted in duplicate and the percentage differences were within 10% for all elements with the exception of zinc (13.9%).

Table B2.2 represents the trace element recovery for the quality assurance samples for the green-lipped mussels of Chapter 4. The mean percentage recoveries of all elements in the standard reference mussel, fish protein (DORM-3) and marine sediments ranged between 90 and 113% with the exception of lead in DORM-3 (Table B2.2). The percentage recovery of lead in DORM-3 was 35.7%, which was comparable with the results obtained in method validation work described in Chapter 2 (Section 2.4.3.2).

Table B2.2: Mean percentage recoveries ± standard error for standard reference materials

Analytes	Mean % recoveries of trace elements		
	Mussel (n = 4)	Fish protein (n = 4)	Sediment (n = 6)
Arsenic	113.1 ± 3.3	97.6 ± 4.7	98.7 ± 2.6
Cadmium	103.1 ± 5.5	104.4 ± 6.8	106.5 ± 5.6
Copper	90.1 ± 1.5	93.0 ± 1.9	90.0 ± 1.1
Iron	99.9 ± 1.5	94.2 ± 2.9	92.7 ± 2.9
Mercury	90.4 ± 5.0	99.0 ± 3.8	95.2 ± 3.5
Lead	99.2 ± 10.7	35.7 ± 1.8	91.7 ± 2.7
Zinc	98.2 ± 2.0	95.8 ± 2.3	90.7 ± 3.7

The percentage differences of the 10th sample duplicate digests were within 10% for all the analytes in shellfish and marine sediments. In addition to the duplicate digestions, every 10th sample was also analysed in duplicate, and every 20th sample was analysed with low level trace element spikes for checking ICP-MS instrument performance and any matrix interferences. The percentage differences of these duplicate analyses ranged from 5 to 9% for all elements in shellfish, and <5% in marine sediments. The percentage recoveries of the spiked elements ranged between 89 and 115% in the green-lipped mussels and from 81 to 117 % in the sediment samples.

B2.3: Trace element concentrations µg L^{-1} (mean ± SE) in the seawater with respect to distance from silt curtain (T1 to T3 combined at sea-fill site) for 8 sampling rounds

Distance	Fe	Cu	Zn	Cd	Pb	
2 m	320 ± 69	0.86 ± 0.39	2.37 ± 1.53	0.023 ± 0.004	0.26 ± 0.06	
20 m	340 ± 44	0.77 ± 0.20	2.17 ± 0.93	0.025 ± 0.006	0.26 ± 0.06	
80 m	347 ± 57	1.09 ± 0.77	1.62 ± 0.15	0.020 ± 0.001	0.26 ± 0.04	
160 m	337 ± 22	0.93 ± 0.43	1.60 ± 0.16	0.020 ± 0.002	0.23 ± 0.02	**Round 1**
400 m	302 ± 24	0.69 ± 0.15	1.69 ± 0.32	0.019 ± 0.001	0.21 ± 0.02	
Reference site	125 ± 15	0.42 ± 0.25	0.72 ± 0.41	0.022 ± 0.001	0.08 ± 0.01	
2 m	576 ± 143	0.76 ± 0.13	4.10 ± 2.1	0.026 ± 0.008	0.55 ± 0.26	
20 m	472 ± 196	0.65 ± 0.19	3.06 ± 1.9	0.023 ± 0.002	0.46 ± 0.33	
80 m	481 ± 115	0.70 ± 0.02	2.87 ± 0.55	0.022 ± 0.001	0.40 ± 0.10	
160 m	405 ± 6	0.63 ± 0.13	2.06 ± 0.26	0.026 ± 0.008	0.30 ± 0.02	**Round 2**
400 m	508 ± 86	0.66 ± 0.06	2.56 ± 0.45	0.022 ± 0.000	0.36 ± 0.07	
Reference site	300 ± 11	0.23 ± 0.02	1.03 ± 0.08	0.011 ± 0.001	0.14 ± 0.01	
2 m	94 ± 25	0.48 ± 0.01	2.01 ± 1.0	0.034 ± 0.017	0.19 ± 0.05	
20 m	94 ± 22	0.39 ± 0.07	1.08 ± 0.69	0.028 ± 0.015	0.15 ± 0.04	
80 m	131 ± 31	0.41 ± 0.04	1.25 ± 0.28	0.029 ± 0.016	0.16 ± 0.03	
160 m	112 ± 15	0.36 ± 0.0	0.65 ± 0.15	0.018 ± 0.003	0.12 ± 0.01	**Round 3**
400 m	131 ± 21	0.41 ± 0.06	0.73 ± 0.37	0.018 ± 0.002	0.12 ± 0.00	
Reference site	185 ± 7	0.22 ± 0.01	0.49 ± 0.06	0.019 ± 001	0.11 ± 0.00	
2 m	246 ± 62	0.49 ± 0.12	1.74 ± 0.53	0.019 ± 0.005	0.33 ± 0.09	
20 m	282 ± 35	0.44 ± 0.07	1.62 ± 0.38	0.018 ± 0.001	0.31 ± 0.09	
80 m	275 ± 21	0.55 ± 0.20	1.40 ± 0.15	0.017 ± 0.001	0.26 ± 0.02	
160 m	253 ± 67	0.44 ± 0.05	1.66 ± 0.46	0.016 ± 0.000	0.26 ± 0.03	**Round 4**
400 m	323 ± 60	0.60 ± 0.21	1.65 ± 0.17	0.017 ± 0.000	0.30 ± 0.04	
Reference site	197 ± 22	0.23 ± 0.02	0.88 ± 0.11	0.019 ± 0.001	0.12 ± 0.01	
2 m	135 ± 16	0.49 ± 0.13	1.79 ± 0.34	0.019 ± 0.005	0.33 ± 0.15	
20 m	168 ± 7	0.46 ± 0.04	1.51 ± 0.11	0.016 ± 0.001	0.33 ± 0.10	
80 m	203 ± 41	0.47 ± 0.04	1.67 ± 0.23	0.017 ± 0.001	0.38 ± 0.08	
160 m	208 ± 50	0.49 ± 0.03	1.58 ± 0.21	0.017 ± 0.001	0.41 ± 0.13	**Round 5**
400 m	182 ± 51	0.46 ± 0.05	1.22 ±0.20	0.016 ± 0.000	0.28 ± 0.07	
Reference site	95 ± 10	0.30 ± 0.05	0.69 ± 0.17	0.015 ± 0.002	0.10 ± 0.01	
2 m	80 ± 14	0.40 ± 0.08	1.65 ± 0.83	0.014 ± 0.002	0.27 ± 0.13	
20 m	79 ± 26	0.98 ± 0.61	1.38 ± 0.67	0.012 ± 0.001	0.24 ± 0.20	
80 m	59 ± 6	0.49 ± 0.28	0.92 ± 0.13	0.013 ± 0.001	0.13 ± 0.02	
160 m	57 ± 11	0.36 ± 0.02	0.78 ± 0.06	0.011 ± 0.000	0.10 ± 0.02	**Round 6**
400 m	72 ± 30	0.35 ± 0.05	0.75 ± 0.10	0.011 ± 0.001	0.15 ± 0.08	
Reference site	109 ± 35	0.21 ± 0.01	0.50 ± 0.10	0.010 ± 0.001	0.12 ± 0.04	
2 m	170 ± 32	0.53 ± 0.08	2.0 ± 0.67	0.012 ± 0.002	0.43 ± 0.02	
20 m	151 ± 28	0.52 ± 0.11	1.36 ± 0.15	0.012 ± 0.000	0.32 ± 0.04	
80 m	159 ± 23	0.51 ± 0.07	1.37 ± 0.43	0.011 ± 0.000	0.30 ± 0.05	
160 m	188 ± 45	0.56 ±0.09	1.53 ± 0.07	0.010 ± 0.000	0.34 ± 0.07	**Round 7**
400 m	160 ± 23	0.51 ± 0.06	1.13 ± 0.16	0.009 ± 0.001	0.28 ± 0.03	
Reference site	194 ± 57	0.26 ± 0.02	0.86 ± 0.19	0.009 ± 0.001	0.14 ± 0.01	
2 m	84 ± 31	0.58 ± 0.05	1.49 ± 0.48	0.013 ± 0.001	0.55 ± 0.25	
20 m	65 ± 3	0.51 ± 0.06	0.93 ± 0.29	0.011 ± 003	0.30 ± 0.07	
80 m	66 ± 5	0.53 ± 0.08	1.25 ± 0.50	0.010 ± 0.001	0.29 ± 0.09	
160 m	71 ± 6	0.51 ± 0.06	0.89 ± 0.18	0.010 ± 0.000	0.27 ± 0.03	**Round 8**
400 m	66 ±13	0.49 ± 0.05	0.74 ± 0.13	0.010 ± 0.000	0.25 ± 0.05	
Reference site	91 ± 5	0.24 ± 0.04	0.45 ± 0.08	0.011 ± 0.001	0.16 ± 0.02	

B3: Trace element concentrations in marine sediments and seawater samples collected around Lyttelton Harbour (sample site 9 was same as the T1A site at the transect line T1 at the sea-fill)

Sample site	Marine sediment µg g dry wt^{-1}.						Seawater µg L^{-1}				
	Fe	Cu	Zn	As	Cd	Pb	Fe	Cu	Zn	Cd	Pb
1	17460.80	4.36	60.52	5.78	0.020	13.08	155.65	0.45	0.73	0.01	0.15
2	18617.00	5.02	53.76	6.20	0.026	14.55	272.39	0.59	1.27	0.02	0.23
3	20818.58	6.94	64.10	6.72	0.025	18.43	448.63	0.87	1.63	0.02	0.35
4	25156.62	9.63	78.82	6.42	0.031	21.55	379.12	0.67	1.52	0.02	0.30
5	20575.12	5.80	58.97	6.55	0.022	16.56	193.26	0.61	1.38	0.02	0.19
6	21012.65	7.70	64.49	6.54	0.028	18.08	212.73	0.53	1.20	0.02	0.19
7	21298.03	5.34	54.34	7.49	0.032	15.31	198.47	0.40	0.96	0.02	0.18
8	18646.79	5.98	49.02	6.03	0.031	16.13	188.81	0.47	1.07	0.03	0.17
9 (T1-A)	19186.87	15.54	151.78	7.24	0.133	75.22	260.98	1.31	4.14	0.03	0.32
10	16358.50	4.68	42.40	5.93	0.025	15.15	124.96	0.40	0.70	0.03	0.11
11	22047.03	8.62	66.44	6.68	0.032	19.08	185.12	0.39	1.05	0.04	0.15
12	22138.12	7.71	60.18	7.73	0.033	17.98	24.28	0.21	0.68	0.03	0.06
13	24733.10	9.94	70.11	7.81	0.041	21.14	83.34	0.25	0.72	0.02	0.10

B4: Pearson's correlation analysis for seawater and marine sediments of Lyttelton Harbour and the reference site (Chapter 4)

Table B4.1: Pearson's correlations coefficients and *P*-values for significant relationships (bold) between trace elements within seawater

Sea-fill site	Fe		Cu		Zn		Cd		Pb	
	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value
Fe	1.000									
Cu	**0.396**	**0.000**	1.000							
Zn	**0.716**	**0.000**	**0.402**	**0.000**	1.000					
Cd	**0.429**	**0.000**	**0.193**	**0.034**	**0.493**	**0.000**	1.000			
Pb	**0.455**	**0.000**	**0.212**	**0.020**	**0.692**	**0.000**	0.095	0.304	1.000	
PG site	**Fe**		**Cu**		**Zn**		**Cd**		**Pb**	
	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value
Fe	1.000									
Cu	-0.117	0.473	1.000							
Zn	**0.628**	**0.000**	0.028	0.865	1.000					
Cd	-0.059	0.719	**0.426**	**0.006**	0.018	0.910	1.000			
Pb	**0.350**	**0.027**	**-0.340**	**0.032**	0.224	0.164	**-0.627**	**0.000**	1.000	

Table B4.2: Pearson's correlations coefficients and *P*-values for significant relationships (bold) between trace elements within sediments

Sea-fill site	**Fe**		**Cu**		**Zn**		**As**		**Cd**		**Pb**	
	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value
Fe	1.000											
Cu	-0.232	0.074	1.000									
Zn	**-0.260**	**0.045**	**0.538**	**0.000**	1.000							
As	**0.490**	**0.000**	-0.234	0.071	-0.081	0.541	1.000					
Cd	**-0.425**	**0.001**	**0.558**	**0.000**	**0.759**	**0.000**	-0.150	0.251	1.000			
Pb	**-0.446**	**0.000**	**0.439**	**0.000**	**0.876**	**0.000**	-0.176	0.179	**0.848**	**0.000**	1.000	

PG site	**Fe**		**Cu**		**Zn**		**As**		**Cd**		**Pb**	
	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value	Correlation value	*P* value
Fe	1.000											
Cu	**0.743**	**0.000**	1.000									
Zn	**0.834**	**0.000**	**0.967**	**0.000**	1.000							
As	**0.937**	**0.000**	**0.764**	**0.000**	**0.853**	**0.000**	1.000					
Cd	0.209	0.377	0.245	0.298	0.168	0.479	0.078	0.742	1.000			
Pb	**0.657**	**0.002**	**0.984**	**0.000**	**0.948**	**0.000**	**0.700**	**0.001**	0.206	0.384	1.000	

Table B4.3: Pearson's correlations coefficients and *P*-values for significant relationships (bold) between seawater trace elements and sediment trace elements

Sea-fill site		Sediment elements									
		Fe		**Cu**		**Zn**		**Cd**		**Pb**	
		Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Water elements	Fe	-0.100	0.449								
	Cu			**0.259**	**0.046**						
	Zn					**0.419**	**0.001**				
	Cd							0.153	0.243		
	Pb									0.169	0.196

PG site		Sediment elements									
		Fe		**Cu**		**Zn**		**Cd**		**Pb**	
		Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Water elements	Fe	0.052	0.827								
	Cu			-0.084	0.724						
	Zn					0.280	0.233				
	Cd							0.098	0.681		
	Pb									0.061	0.799

B5: Trace element concentrations µg g dry wt^{-1} (mean ± SE) in the marine sediments at the sea-fill site with respect to distance from silt curtains (T1, T2 and T3 combined) for the four sediment sampling rounds (Chapter 4)

Distance from silt curtain	Fe	Cu	Zn	As	Cd	Pb
Sediment sampling round 1 (R1) (April 2012)						
2 m	23028 ±3340	12.8 ± 2.9	101 ± 44	7.8 ± 0.8	0.087 ± 0.04	42.0 ± 29
20 m	27069 ± 3611	15.4 ± 6.5	83.5 ± 7.5	7.8 ± 0.9	0.067 ±0.02	26.2 ± 4.8
80 m	26064 ± 2402	12.4 ± 0.6	81.2 ± 7.7	7.5 ± 0.5	0.062 ± 0.01	28.2 ± 4.8
160 m	23076 ± 4307	12.0 ± 2.0	78.5 ± 18	8.4 ± 0.7	0.060 ± 0.01	26.7 ± 3.5
400 m	25065 ± 1914	12.3 ± 0.2	81.6 ± 5.2	7.5 ± 0.3	0.053 ± 0.01	23.7 ± 0.8
Reference site	18761 ± 1925	6.1 ±1.2	51.5 ± 7.6	7.6 ± 0.4	0.037 ± 0.00	15.3 ± 2.1
Sediment sampling round 2 (R3) (October 2012)						
2 m	23448 ± 3199	13.1 ± 0.8	97.9 ± 31	8.8 ± 1.6	0.081 ± 0.04	40.1 ± 24
20 m	25392 ± 1434	12.6 ± 0.5	85.8 ± 9.3	8.8 ± 0.4	0.064 ± 0.01	28.2 ± 4.0
80 m	25352 ± 2392	11.2 ± 2.1	80.4 ± 8.1	9.1 ± 1.1	0.079 ± 0.03	26.9 ± 4.6
160 m	25385 ± 1452	11.6 ± 0.7	79.1 ± 8.6	8.7 ± 0.6	0.054 ± 0.01	24.1 ± 2.2
400 m	24658 ± 1473	11.6 ± 0.3	76.5 ± 9.9	7.9 ± 0.7	0.059 ± 0.02	22.9 ± 1.0
Reference site	19001 ± 1366	6.0 ±0.8	50.7 ± 4.8	7.6 ± 0.6	0.037 ± 0.00	14.9 ± 1.6
Sediment ampling round 3 (R5) (April 2013)						
2 m	26253 ± 1747	11.01 ± 1.5	84.3 ± 13	9.2 ± 1.0	0.067 ± 0.01	30.4 ± 5.1
20 m	27799 ± 1840	11.6 ± 1.8	83.4 ± 8.8	9.5 ± 1.2	0.059 ± 0.01	26.9 ± 4.7
80 m	25544 ± 334	11.4 ± 1.2	77.3 ± 12	8.7 ± 0.8	0.055 ± 0.01	24.7 ± 2.9
160 m	26936 ± 1597	11.8 ± 0.9	80.4 ± 9.2	8.8 ± 0.3	0.062 ±0.00	26.5 ± 2.8
400 m	24107 ± 3876	10.8 ± 1.3	72.1 ± 14	7.4 ± 1.0	0.047 ± 0.00	20.7 ± 3.2
Reference site	26104 ± 2258	7.7 ± 1.4	61.9 ± 9.4	8.9 ± 0.8	0.060 ± 0.04	18.0 ± 2.8
Sediment sampling round 4 (R7) (October 2013)						
2 m	27648 ±5456	11.2 ± 1.9	91.8 ± 13	9.3 ± 0.9	0.064 ± 0.02	34.5 ±19
20 m	28994 ± 708	10.6 ± 1.8	82.2 ± 10	9.8 ± 0.4	0.062 ± 0.01	26.4 ± 4.9
80 m	29179 ± 2663	10.0 ± 1.0	73.9 ± 8.1	8.7 ± 0.3	0.052 ± 0.10	23.2 ± 4.01
160 m	27736 ± 1149	10.1 ± 0.9	73.2 ± 8.3	8.9 ± 0.2	0.049 ± 0.00	20.8 ± 1.5
400 m	28019 ± 2020	10.5 ± 0.5	76.1 ± 7.2	8.6 ± 0.5	0.045 ± 0.01	22.8 ± 0.1
Reference site	27151 ± 1220	6.8 ± 0.8	59.7 ± 5.4	9.5 ± 0.7	0.035 ± 0.00	16.0 ± 1.6

B6: Organic content of sediments from the four transect lines for the first two sediment sampling rounds (R1 and R3), presented as a weight percentage (Chapter 4)

Sample	wt % of organic content	Transect line	Sampling round	Distance from silt curtain (m)
1A-1	7.08	1	1	2
1B-1	8.25	1	1	20
1C-1	6.65	1	1	80
1D-1	6.00	1	1	160
1E-1	5.29	1	1	400
1A-2	5.13	1	2	2
1B-2	5.61	1	2	20
1C-2	5.37	1	2	80
1D-2	5.02	1	2	160
1E-2	5.08	1	2	400
2A-1	4.90	2	1	2
2B-1	4.31	2	1	20
2C-1	4.80	2	1	80
2D-1	5.34	2	1	160
2E-1	5.30	2	1	400
2A-2	4.42	2	2	2
2B-2	5.00	2	2	20
2C-2	4.84	2	2	80
2D-2	5.14	2	2	160
2E-2	5.38	2	2	400
3A-1	11.53	3	1	2
3B-1	7.39	3	1	20
3C-1	4.68	3	1	80
3D-1	4.64	3	1	160
3E-1	4.25	3	1	400
3A-2	5.72	3	2	2
3B-2	5.43	3	2	20
3C-2	4.63	3	2	80
3D-2	4.67	3	2	160
3E-2	6.48	3	2	400
4A-1	3.75	4	1	2
4B-1	4.12	4	1	20
4C-1	4.67	4	1	80
4D-1	4.88	4	1	160
4E-1	5.33	4	1	400
4A-2	4.44	4	2	2
4B-2	5.34	4	2	20
4C-2	5.61	4	2	80
4D-2	6.70	4	2	160
4E-2	7.11	4	2	400

B7: Trace element concentrations µg g wet wt^{-1} (mean ± SE) in the green-lipped mussels collected at the sea-fill site and reference site (Chapter 4)

Sampling date (round)	Fe	Cu	Zn	As	Cd	Hg	Pb
Lyttelton Harbour sea-fill site (Battery Point-BP)							
April 2012 (R1)	140.2 ± 36.7	0.89 ± 0.11	18.1 ± 2.4	2.43 ± 0.34	0.076 ± 0.026	0.022 ± 0.005	0.34 ± 0.09
July 2012 (R2)	125.0 ± 30.2	0.97 ± 0.15	18.2 ± 2.3	2.36 ± 0.14	0.079 ± 0.023	0.022 ± 0.005	0.41 ± 0.09
January 2013 (R4)	95.5 ± 27.0	0.88 ± 0.26	14.8 ± 3.3	1.95 ± 0.22	0.069 ± 0.013	0.028 ± 0.005	0.23 ± 0.05
April 2013 (R5)	148.8 ± 23.5	0.79 ± 0.07	16.0 ± 1.9	2.01 ± 0.11	0.060 ± 0.011	0.034 ± 0.003	0.28 ± 0.04
July 2013 (R6)	119.6 ± 8.5	0.82 ± 0.04	15.9 ± 1.2	2.01 ± 0.05	0.069 ± 0.018	0.034 ± 0.003	0.31 ± 0.02
November 2013 (R7)	123.0 ± 15.6	0.88 ± 0.07	13.6 ± 1.2	2.20 ± 0.11	0.064 ± 0.016	0.029 ± 0.004	0.38 ± 0.03
January 2014 (R8)	124.2 ± 23.3	0.90 ± 0.07	16.1 ± 3.7	2.56 ± 0.19	0.071 ± 0.020	0.020 ± 0.004	0.30 ± 0.06
Reference site (Pigeon Bay - PG)							
April 2012 (R1)	74.4 ± 11.8	0.73 ± 0.08	13.9 ± 2.0	2.43 ± 0.43	0.096 ± 0.029	0.016 ± 0.003	0.07 ± 0.01
July 2012 (R2)	71.1 ± 13.3	0.74 ± 0.11	12.4 ± 1.7	1.82 ± 0.22	0.086 ± 0.025	0.017 ± 0.003	0.07 ± 0.01
January 2013 (R4)	78.2 ± 16.1	0.78 ± 0.11	13.2 ± 2.0	1.94 ± 0.24	0.097 ± 0.029	0.021 ± 0.004	0.08 ± 0.02
April 2013 (R5)	84.6 ± 20.4	0.74 ± 0.12	14.1 ± 3.5	1.64 ± 0.22	0.099 ± 0.022	0.031 ± 0.004	0.10 ± 0.02
July 2013 (R6)	81.9 ± 12.0	0.65 ± 0.05	12.9 ± 1.6	1.63 ± 0.07	0.104 ± 0.017	0.022 ± 0.004	0.10 ± 0.01
November 2013 (R7)	65.3 ± 10.4	0.67 ± 0.09	11.7 ± 1.3	1.95 ± 0.20	0.072 ± 0.008	0.018 ± 0.004	0.09 ± 0.01
January 2014 (R8)	90.4 ± 8.3	0.78 ± 0.10	15.7 ± 2.4	2.67 ± 0.45	0.083 ± 0.023	< LOQ	0.08 ± 0.01

B8: Pearson's correlations coefficients and *P*-values for significant relationships (bold) between seawater trace elements and green mussel trace elements (Chapter 4)

Sea-fill site		**Green Mussel elements**									
		Fe		Cu		Zn		Cd		Pb	
		Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Seawater elements	Fe	0.0028	0.995								
	Cu			0.491	0.263						
	Zn					0.591	0.163				
	Cd							0.592	0.161		
	Pb									0.385	0.394
PG site		**Green Mussel elements**									
		Fe		Cu		Zn		Cd		Pb	
		Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Seawater elements	Fe	-0.552	0.199								
	Cu			0.057	0.903						
	Zn					-0.407	0.365				
	Cd							0.294	0.522		
	Pb									-0.539	0.211

B9: Pearson's correlations coefficients and *P*-values for significant relationships (bold) between green-lipped mussel trace elements and sediment trace elements

Sea-fill site		Green Mussel elements											
		Fe		Cu		Zn		As		Cd		Pb	
		Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Sediment elements	Fe	-0.941	0.219										
	Cu			0.221	0.858								
	Zn					0.979	0.132						
	As							-0.827	0.380				
	Cd									0.994	0.072		
	Pb											0.820	0.388

PG site		Green Mussel elements											
		Fe		Cu		Zn		As		Cd		Pb	
		Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value	Correlation coefficient	*p* value
Sediment elements	Fe	0.878	0.326										
	Cu			0.923	0.250								
	Zn					0.111	0.929						
	As							-0.354	0.770				
	Cd									-0.851	0.353		
	Pb											0.915	0.264

APPENDIX - C

C1: Percentage recoveries of trace elements in the quality assurance samples

Table C1.1 represents the percentage recoveries of trace elements in the quality assurance samples digested with the biota samples of Chapter 5. The mean percentage recoveries of trace elements in the standard reference materials are provided in Table C1.1

Table C1.1: Mean percentage recoveries ± standard error for standard reference materials

Analytes	Mean % recoveries of trace elements in standard reference materials			
	Mussel	Fish protein	Bovine liver	Tomato leaves
	(n = 6)	(n = 6)	(n = 6)	(n = 2)
Arsenic	116.4 ± 4.8	97.6 ± 6.2	< LOQ	< LOQ
Cadmium	104.7 ± 2.3	101.8 ± 2.6	98.2 ±5.6	93.8 ± 3.7
Copper	90.1 ± 4.4	90.2 ± 2.2	89.8 ± 4.3	100.5 ± 1.7
Iron	103.9 ± 6.4	96.0 ± 2.3	93.0 ± 9.6	98.7 ± 1.0
Mercury	91.7 ± 11.2	90.6 ± 8.3	< LOQ	< LOQ
Lead	97.1 ± 5.2	36.4 ± 4.0	88.5 ± 6.2	NP*
Zinc	101.7 ± 6.4	97.0 ± 8.6	92.7 ± 3.6	95.3 ± 1.7

***NP- not provided in the certified values; LOQ- limit of quantification**

The percentage differences of the duplicate digests were less than 10% for all analytes in fish muscle, fish organs, crab muscle, shellfish, polychaete worms and green algae samples. The 10th sample duplicate analyses were <12% for all elements in fish muscle (although most elements were <5%), and <10% for all elements in crab muscle, shellfish, polychaete worms and green algae. The 20th sample spike analysis had percentage recoveries of 89 to 120% for all samples with the exception of arsenic spiked in polychaete worms (126%) and green algae (130%). The percentage recoveries of the spiked elements in fish organs ranged from 81 to 119%.

C2: Trace element concentrations (µg g wet wt^{-1}.) measured in fish muscle and organs from Lyttelton Harbour and Pigeon Bay (Chapter 5)

Fish samples	Trace element concentrations (mean ± SE) in fish muscle and organs						
	Total As	Cd	Cu	Fe	Hg	Pb	Zn
Lyttelton Harbour fish							
Spotty (*Notolabrus celidotus*)							
Muscle (n = 12)	2.74± 0.97	<LOQ	0.12±0.02	1.33±0.49	0.07±0.02	<LOQ	4.99±2.61
Liver (n = 12)	8.12±3.81	0.09±0.07	2.86±0.71	243.37±60.42	0.11±0.04	0.05±0.03	21.08±4.32
Kidney (n = 12)	11.00±10.49	0.06±0.05	1.45±0.58	296.28±152.51	0.27±0.17	0.08±0.10	22.13±8.79
Wrasse (*Notolabrus fucicola*)							
Muscle (n = 12)	1.61±0.90	<LOQ	0.13±0.02	2.50±0.78	0.28±0.08	<LOQ	4.32
Liver (n = 12)	6.68±5.99	1.53±0.89	3.21±0.91	225.62±70.01	1.62±0.92	0.21±0.12	26.66±7.60
Kidney (n = 12)	3.87±3.12	0.24±0.07	0.93±0.12	545.89±126.02	1.07±0.37	0.11±0.07	16.47±1.10
Gonad (n = 12)	4.64±3.30	0.03±0.02	1.23±0.38	74.81±64.55	0.40±0.22	0.02±0.02	129.05±67.82
Pigeon Bay Fish							
Spotty (*Notolabrus celidotus*)							
Muscle (n = 12)	2.76±0.86	<LOQ	0.12±0.01	1.62±0.48	0.06±0.03	<LOQ	5.29±0.53
Liver (n = 12)	9.93±3.88	0.34±0.21	9.17±8.60	233.74±68.47	0.12±0.03	0.02±0.02	32.29±8.60
Kidney (n = 12)	4.13±4.64	0.04±0.02	0.72±0.38	110.39±81.97	0.08±0.05	0.01±0.01	11.70±7.41
Wrasse (*Notolabrus fucicola*)							
Muscle (n = 12)	2.42±1.39	<LOQ	0.13±0.02	2.29±0.83	0.22±0.08	<LOQ	3.36±0.28
Liver (n = 12)	6.27±4.67	2.68±1.37	4.99±2.10	226.34±78.03	0.70±0.51	0.06±0.03	26.47±5.23
Kidney (n = 12)	3.06±1.64	0.51±0.28	1.06±0.16	798.75±464.05	0.53±0.26	0.03±0.01	16.71±1.42
Gonad (n = 12)	4.60±2.03	0.05±0.06	1.23±0.42	90.94±78.35	0.29±0.17	0.01±0.01	133.25±70.25

LOQ-Limit of quantification, SE – standard error, n- number of replicates

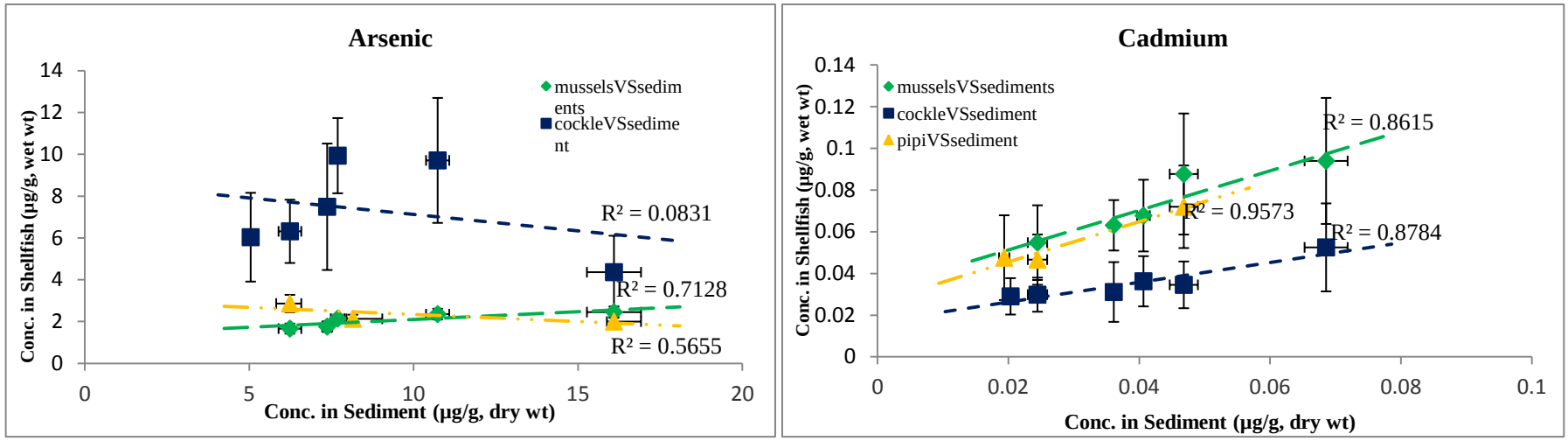
C3: Trace element concentrations (µg g^{-1} wet wt) measured in crab, shellfish, and marine worm and green algae samples (Chapter 5)

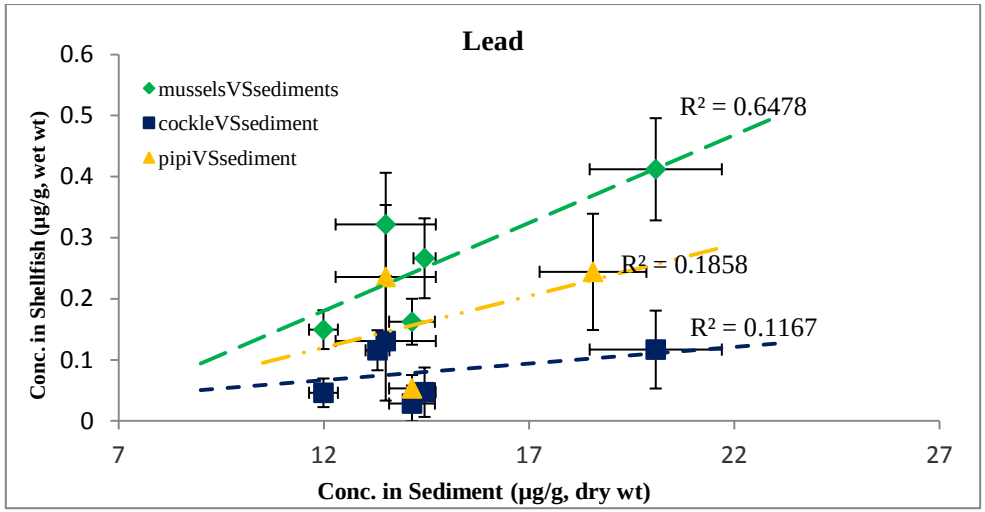
Biota samples	Trace element concentrations (mean ± SE) in biota						
	Total As	Cd	Cu	Fe	Hg	Pb	Zn
Lyttelton Harbour							
Cancer crab (*Metacarcinus novaezelandiae*) (n = 12)	11.7±4.47	<LOQ	9.27±4.01	6.56±1.84	0.07±0.03	0.01±0.00	63.96±3.65
Green-lipped mussel (*Perna canaliculus*) (n = 12)	2.02±0.10	0.06±0.01	0.79±0.06	147.8±22.29	0.03±0.00	0.28±0.04	15.81±1.76
Polychaete worm (n = 12)	16.39±0.29	0.75±0.02	1.24±0.03	86.73±5.37	0.01±0.00	0.12±0.00	41.63±7.86
Green algae (*Ulva* sp.*)* (n = 12)	1.46±0.12	0.01±0.00	1.69±0.14	1577.15±136.01	<LOQ	0.96±0.11	6.31±0.59
Pigeon Bay							
Cancer crab (*Metacarcinus novaezelandiae*) (n = 12)	10.86±5.68	<LOQ	9.62±2.84	4.44±0.91	0.07±0.03	<LOQ	62.1±6.25
Green lipped mussel (*Perna canaliculus*) (n = 12)	1.63±0.22	0.09±0.02	0.72±0.11	83.4±21.86	0.03±0.00	0.1±0.02	13.33±3.59
Polychaete worm (n = 12)	4.87±0.11	0.35±0.01	0.76±0.02	49.56±1.40	<LOQ	0.04±0.00	26.3±7.80
Green algae (*Ulva* sp.*)* (n = 12)	2.31±1.07	0.01±0.00	1.26±0.74	768±183.94	<LOQ	0.41±0.10	2.84±0.72

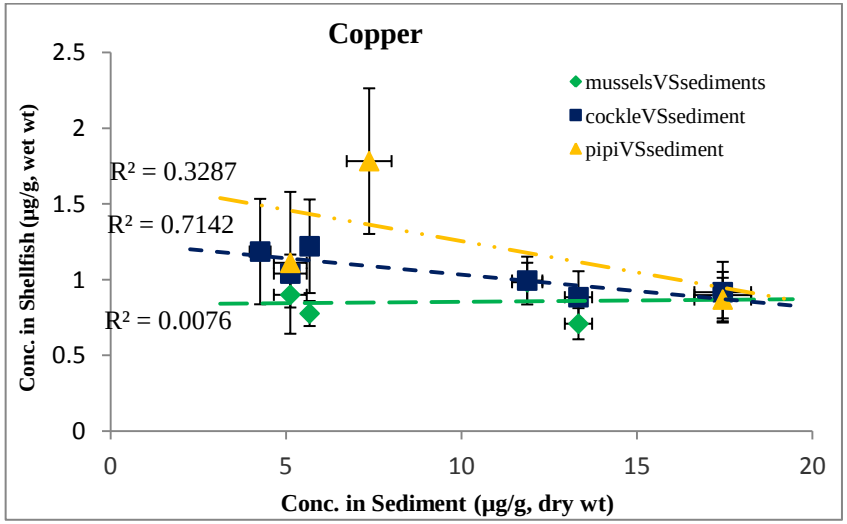
LOQ-Limit of quantification, SE – standard error, n- number of replicates

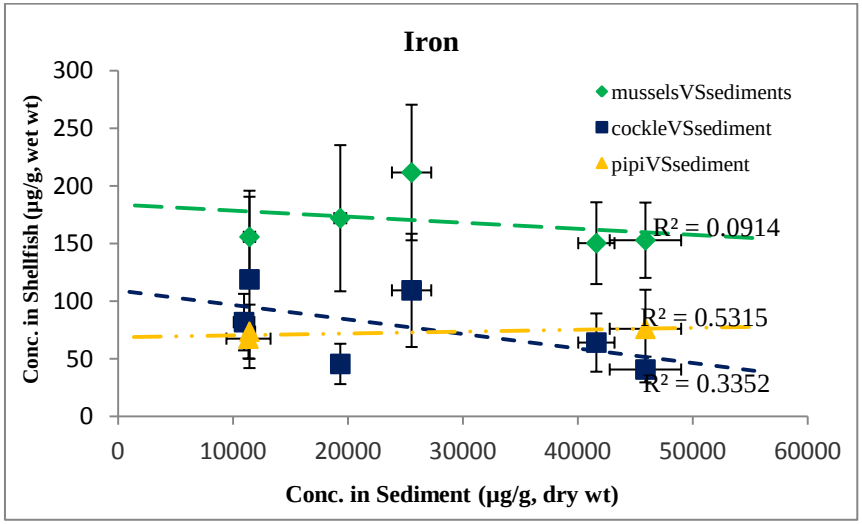
APPENDIX - D

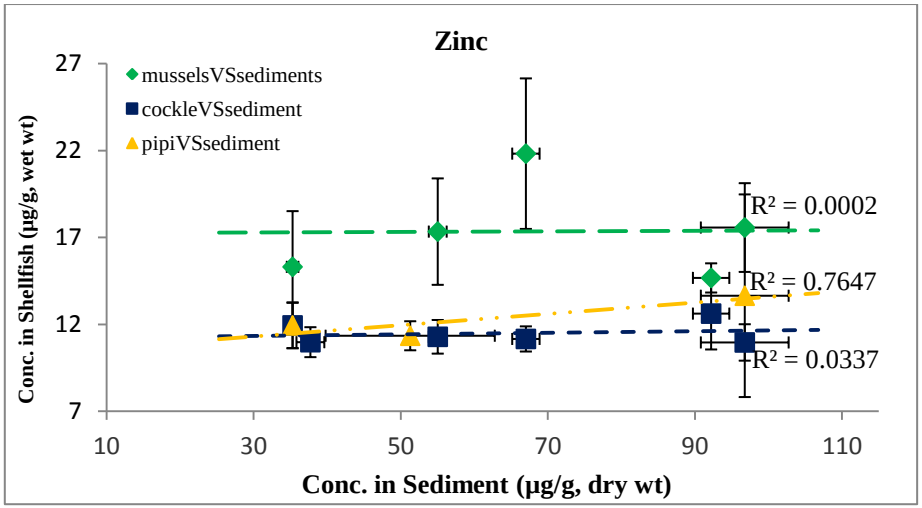
D1. Relations between trace elements in shellfish and associated sediments (Chapter 6)




Lead
musselsVSsediments
cockleVSsediment
pipiVSsediment
R² = 0.6478
R² = 0.1858
R² = 0.1167
Conc. in Shellfish (µg/g, wet wt)
Conc. in Sediment (µg/g, dry wt)
0
0.1
0.2
0.3
0.4
0.5
0.6
7
12
17
22
27


Copper
musselsVSsediments
cockleVSsediment
pipiVSsediment
R² = 0.3287
R² = 0.7142
R² = 0.0076
Conc. in Shellfish (µg/g, wet wt)
Conc. in Sediment (µg/g, dry wt)
0
0.5
1
1.5
2
2.5
0
5
10
15
20


Iron
musselsVSsediments
cockleVSsediment
pipiVSsediment
R² = 0.0914
R² = 0.5315
R² = 0.3352
Conc. in Shellfish (µg/g, wet wt)
Conc. in Sediment (µg/g, dry wt)
0
50
100
150
200
250
300
0
10000
20000
30000
40000
50000
60000


Zinc
musselsVSsediments
cockleVSsediment
pipiVSsediment
R² = 0.0002
R² = 0.7647
R² = 0.0337
Conc. in Shellfish (µg/g, wet wt)
Conc. in Sediment (µg/g, dry wt)
27
22
17
12
7
10
30
50
70
90
110

D2: Pearson correlation coefficients for statistically significant correlations between trace elements within the same shellfish species, and within sediments (Chapter 6)

Mussel	**Fe**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**
Fe	1						
Cu	0.598	1					
Zn	**0.923***	0.721	1				
As	-0.111	-0.065	0.119	1			
Cd	-0.376	-0.431	-0.209	**0.926***	1		
Hg	0.848^	0.531	0.651	-0.612	-0.787	1	
Pb	0.828^	0.700	0.670	-0.549	-0.781	**0.967****	1
Cockle	**Fe**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**
Fe	1						
Cu	0.001	1					
Zn	0.157	-0.457	1				
As	0.302	-0.189	0.496	1			
Cd	-0.204	-0.712	**0.732^**	0.612	1		
Hg	0.248	0.263	-0.109	0.718	-0.005	1	
Pb	**0.935****	0.296	-0.080	0.132	-0.444	0.200	1
Pipi	**Fe**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**
Fe	1						
Cu	**-0.999***	1					
Zn	0.876	-0.860	1				
As	0.089	-0.121	-0.402	1			
Cd	0.705	-0.681	0.959	-0.644	1		
Hg	-0.616	0.590	-0.919	0.730	**-0.993^**	1	
Pb	-0.7537	0.732	-0.977	0.587	**-0.997***	0.982	1
Sediment	**Fe**	**Cu**	**Zn**	**As**	**Cd**	**Hg**	**Pb**
Fe	1						
Cu	**0.938****	1					
Zn	**0.979*****	**0.951*****	1				
As	**0.885****	**0.912****	**0.891****	1			
Cd	**0.888****	**0.743^**	**0.869***	0.608	1		
Pb	-0.247	0.044	-0.110	-0.136	-0.317		1

p-value, ^ <0.1, *<0.05, **<0.01, ***<0.001