

**Heavy metal concentrations in the Pacific oyster;  
*Crassostrea gigas*.**

**Percy Perera**

**Thesis submitted in partial fulfilment of the degree of Master of Applied Science**

**Auckland University of Technology  
Auckland**

**September 2004**

## **Acknowledgement**

I would like to sincerely thank my supervisor Dr. Andrea C. Alfaro for her guidance, patience throughout this work. I am especially thankful to Professor John Buckeridge for his willingness showed while editing this thesis.

I would like to thank my colleagues Nabil Soliman, Claire Barnaby and Peter Conway. Your friendship and advice has been most appreciated.

Thank you Christopher Whyburd and Yan Wang for your contribution in the laboratory. Thanks for Dr. Pat Strauss for her suggestions generously given during the writing of this thesis.

Finally I would like to thank my wife, and two sons for their continual love and support. I could not have managed without you.

## Abstract

Heavy metals present in high concentrations in aquatic habitats are bioaccumulated within the tissues of intertidal organisms. The chemical analyses of animal tissues and sediments provide an indication of bioavailability of heavy metals in the environment. Monitoring of the coastal pollution using organisms is widely practiced all over the world.

Chemical analysis of the tissues of Pacific oyster, *Crassostrea gigas*, and river sediments were used in this study to monitor the environmental concentrations, of cadmium, copper, lead and zinc of shallow coastal areas near to the river mouths of Mahurangi, Awaruku, Waiake, Taiorahi and Wairau. All of these river mouths are situated along the northeastern coast of Auckland. Each month, during the period of November 2002 to October 2003, three replicate samples of oysters, and sediments were collected from each of the river mouths for analysis. Three additional replicate samples of oysters were collected separately, in each month in order to calculate the condition index of oysters. Oyster tissues and sediments were analysed with Inductively Coupled Atomic Plasma Emission Spectrometer to detect the concentration levels of cadmium, copper, lead and zinc.

The highest level of copper and zinc concentrations in the oyster tissues was observed in the river mouth of Wairau. Wairau river mouth receives water from heavily urbanised and industrialised catchments. A higher concentration of cadmium was observed in the oysters of the river mouth of Mahurangi than in the oysters in the other sites. The reason for this difference could be due to the heavy use of cadmium contaminated fertilizers at the pasture lands situated around the Mahurangi estuary. Therefore, the relationship between the land use of the catchments and the degree of pollution of the estuarine habitats could be established from the data obtained from this study. Higher concentrations of heavy metals were found in the sediments of Waiake, Taiorahi and Wairau compared to the sediments of Mahurangi and Awaruku. Significantly higher level of copper was observed in the sediments of Awaruku. However no clear co-relation was found between heavy metal concentration in oysters

and in sediments. Variations of the condition of oysters were closely related to seasonal changes of the life cycle of the oysters. No clear relationship was found between the condition of the oysters and the heavy metal concentration of the river mouth habitats.

This study provides evidence that Pacific oysters are good organisms to use as bioindicators of environmental heavy metal levels in shallow coastal waters. The results of this study suggest a clear relationship between the heavy metal concentration in river waters and the land use of the catchment areas of those rivers. The results may be useful in management strategies of the northeastern coastal areas of Auckland.

## Table of Contents

Title page .....	i
Acknowledgments.....	ii
Abstract.....	iii
Table of contents.....	v
List of figures.....	viii
List of tables.....	x
Appendix 1: results of t- test of AGAL – 10 and the TAE certified	
Values.....	xi
Statement of originality.....	xii
Chapter1-.....	1
1.General Introduction.....	1
1.1 Pollution of marine environment.....	1
1.1.1Effects of stormwater on marine pollution.....	2
1.1.2 Effects of atmospheric air on marine pollution. ....	3
1.1.3Effects of dumping of wastes on marine habitats.. ....	3
1.2Heavy metal pollution in marine environments.....	4
1.2.1Heavy metal pollution in coastal environments.....	5
1.2.2Heavy metal pollution in New Zealand coastal environments .....	6
1.2.3Sources of heavy metal pollution in coastal environments .....	6
1.3 Effects of heavy metals on animals.....	7
1.3.1Effects of heavy metals on human health.....	9
1.4 Importance of the management of the coastal environment .....	10
1.5 Monitoring of coastal environment .....	11
1.5.1Ecological monitoring of the coastal environment.....	11
1.5.2Biological monitoring of the coastal environment.....	12
1.5.3 Monitoring the coastal environment using chemical methods. ....	12
1.5.3.1Chemical analysis of water. ....	13
1.5.3.2Chemical analysis of sediments. ....	13
1.5.3.3 Chemical analysis of animal tissues.....	14

1.6	Bioaccumulation of heavy metals in coastal animals.....	16
1.6.1	Quantifying the bioaccumulation.....	16
1.6.2	Factors influencing the bioaccumulation of heavy metals in animals .....	17
1.6.3	Effects of Bioaccumulation in ivalves.....	20
1.7	Condition Index of oysters.....	22
1.7.1	Measurement of CI.....	23
1.7.2	Factors affecting condition.....	23
1.8	Use of animals as biomonitors to detect the heavy metal concentration in coastal habitats.....	24
1.8.1	Use of bivalves as biomonitors .....	26
1.9	Heavy metal pollutants in sediment.....	27
1.9.1	Relationship of heavy metal content between sediments and the organisms.....	28
1.9.2	Factors effecting heavy metal adsorption of sediments .....	28
1.9.3	Disadvantages of sediment analyses .....	29
1.10	Study aim .....	30
1.11	Summary .....	30
	Chapter 2 .....	31
2.	Materials and methods .....	31
2.1.	Study sites .....	31
2.2	Sampled organism; Pacific oyster .....	34
2.2.1	Morphology .....	34
2.3	Advantages of selecting <i>Crassostrea gigas</i> for this study.....	35
2.4	Sampling design.....	36
2.5	Statistical analysis.....	36
2.6	Collecting of samples.....	36
2.6.1	Sampling of oysters for analysis of heavy metals.....	36
2.6.1.1	Preparation of oysters for chemical analysis.....	37
2.6.1.2	Chemical analysis of oyster tissues.....	37
2.6.2	Sampling of oysters for condition measurements.....	39
2.6.2.1	Preparation of oysters for condition indices measurements.....	39
2.6.3	Sediment sampling .....	40
2.6.3.1	Preparation of Sediments for analysis.....	40

2.6.3.2 Chemical analysis of sediments.....	41
2.7 Reagents used for the chemical analysis.....	41
2.8 Summary .....	41
Chapter 3 .....	42
3. Results .....	42
3.1. Results of the chemical analysis of the oyster tissues .....	42
3.1.1 Spatial and temporal variations of cadmium concentrations in oyster tissues .....	43
3.1.2 Spatial and temporal variations of copper concentrations in oyster tissue .....	46
3.1.3 Spatial and temporal variations of lead concentrations in oyster tissues .....	49
3.1.4 Spatial and temporal variations of zinc concentrations in oyster tissues....	52
3.2 Results of the chemical analysis of the sediments .....	55
3.2.1 Spatial and temporal variations of cadmium concentrations in sediments.....	55
3.2.2 Spatial and temporal variations of copper concentrations in sediments.....	59
3.2.3 Spatial and temporal variations of lead concentrations in sediments.....	62
3.2.4 Spatial and temporal variations of zinc concentrations in sediments.....	66
3.3 Results of condition index (CI) measurements.....	70
Chapter 4 .....	74
4. Discussion .....	74
4.1 Variation of heavy metals in oysters and in sediments .....	74
4.2 variation of Condition Index of oysters.....	84
4.3 Summary .....	88
Chapter 5 .....	90
5. Conclusions .....	90

## List of Figures

Figure 2.1 Location of study sites at Mahurangi, Awaruku, Waiake, Taiorahi and Wairau. ....	33
Figure 2.2 Outer surface view of the top shell and the inner surface view of the bottom hell of <i>Crassostrea gigas</i> .....	35
Figure 3.1 Monthly variation of cadmium concentration in oyster tissues at the five studied river mouths during November 2002 to October 2003.....	43
Figure 3.2 Mean $\pm$ SD of annual cadmium concentration in oyster tissues sampled at the five river mouths during the period of November 2002 to October 2003.....	43

Figure 3.3	Monthly variation of copper concentration in oyster tissues at the five studied river mouths during November 2002 to October 2003.....	46
Figure 3.4	Mean $\pm$ SD of annual copper concentration in oyster tissues sampled at the five river mouths during the period of November 2002 to October 2003.....	46
Figure 3.5	Monthly variation of lead concentration in oyster tissues at the five studied river mouths during November 2002 to October 2003.....	49
Figure 3.6	Mean $\pm$ SD of annual lead concentration in oyster tissues sampled at the five river mouths during the period of November 2002 to October 2003.....	49
Figure 3.7	Monthly variation of zinc concentration in oyster tissues at the five studied river mouths during November 2002 to October 2003.....	52
Figure 3.8	Mean $\pm$ SD of annual zinc concentration in oyster tissues sampled at the five river mouths during the period of November 2002 to October 2003.....	52
Figure 3.9	Monthly variation of cadmium concentration in sediments at the five studied river mouths during November 2002 to October 2003.....	55
Figure 3.10	Mean $\pm$ SE of annual cadmium concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.....	55
Figure 3.11	Variation of mean annual $\pm$ SD of cadmium concentration in oyster tissues and sediments sampled at the five river mouths during the period of November 2002 to October 2003.....	56
Figure 3.12	Monthly variation of copper concentration in sediments at the five studied river mouths during November 2002 to October 2003.....	59
Figure 3.13	Mean $\pm$ SD of annual copper concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.....	59
Figure 3.14	Variation of mean annual $\pm$ SD of copper concentration in oyster tissues and sediments sampled at the five river mouths during the period of November 2002 to October 2003.....	60
Figure 3.15	Monthly variation of lead concentration in sediments at the five studied river mouths during November 2002 to October 2003.....	62



Figure 3.16 Mean $\pm$ SD of annual lead concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.....	62
Figure 3.17 Variation of mean annual $\pm$ SD of lead concentration in oyster tissues and sediments sampled at the five river mouths during the period of November 2002 to October 2003.....	63
Figure 3.18 Monthly variation of zinc concentration in sediments at the five studied river mouths during November 2002 to October 2003.....	66
Figure 3.19 Mean $\pm$ SD of annual zinc concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.....	66
Figure 3.20 Variation of mean annual $\pm$ SD of zinc concentration in oyster tissues and sediments at the five river mouths during the period of November 2002 to October 3.....	67
Figure 3.21 Monthly variations of CI at the five river mouth habitats between November 2002 and October 2003.....	70
Figure 3.22 Variation $\pm$ SD of CI and mean annual cadmium concentration in oysters at five river mouths between November 2002 and October 2003.....	71
Figure 3.23 Variation $\pm$ SD of CI and mean annual copper concentration in oysters at five river mouths between November 2002 and October 2003.....	71
Figure 3.24 Variation $\pm$ SD of CI and mean annual lead concentration in oysters at five river mouths between November 2002 and October 2003.....	72
Figure 3.25 Variation $\pm$ SD of CI and mean annual zinc concentration in oysters at five river mouths between November 2002 and October 2003.....	72

## List of Tables

Table 1.1: Relationship between grain size and concentration of Copper, Lead and Zinc .....	29
Table 2.1: Table 2.1 Concentration values of AGAL – 10 obtained in this study and the TAE certified values .....	38
Table 3.1: Results of analysis of variance for cadmium concentration between sites.....	44
Table 3.2: Tukey's comparison of means for cadmium.....	45

Table 3.3: Results of analysis of variance for copper concentration between sites	47
Table 3.4: Tukey's comparison of means for copper...	48
Table 3.5: Results of analysis of variance for lead concentration between sites	50
Table 3.6: Tukey's comparison of means for	51
Table 3.7: Results of analysis of variance for zinc concentration between sites	53
Table 3.8: Tukey's comparison of means for zinc	54
Table 3.9: Results of analysis of variance for cadmium concentration in sediments between sites	57
Table 3.10: Tukey's comparison of means for cadmium in sediments	58
Table 3.11: Results of analysis of variance for copper concentration in sediments between..... sites	61
Table 3.12: Tukey's comparison of means for copper	61
Table 3.13: Analysis of variance for lead concentration in sediments between sites	64
Table 3.14: Tukey's comparison of means for lead	65
Table 3.15: Results of analysis of variance for zinc concentration in sediments between Sites	68
Table 3.16: Tukey's comparison of means for zinc	69
Table 4.1 : Permitted maximum concentration levels of metal contaminant in Molluscs	75

**Appendix 1:** Results of t- test of AGAL – 10 and the TAE certified values ..... 93

## **Statement of originality**

‘I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made in the acknowledgements’

.....

.....

# **Chapter One**

## **1 General Introduction**

### **1.1 Pollution of marine environment**

As two thirds of the Earth's surface is covered by the oceans, pollution of the marine environment has a great impact on marine habitats. Dumping of wastes, maritime transport and extensive run-off from farm lands can result in high levels of pollution in marine habitats.

In 1990, the Joint Group of Experts on Scientific Aspects of Marine Pollution (GESAMP) defined marine pollution as the direct or indirect introduction of substances or energy into the marine environment, resulting in deleterious effects to living resources, and human health (Clark 1997; Gaw 1997).

Marine pollution has become a major environmental problem all over the world. Extensive research is being undertaken to minimize the effect of marine pollution on the global environment. As coastal waters are very sensitive to contaminants from natural streams and stormwater outlets (Penny 1984), coastal habitats are more susceptible to the impact of pollution than other marine habitats.

Pollutants reach the marine environment either by direct drainage of wastes or by river runoffs. River runoff gathers terrestrial pollutants mainly from catchment areas and transports them to coastal habitats. River runoffs have been identified as a major source of coastal pollution around the world (Clark 1997).

River water contains different concentrations of various pollutants. The types and amounts of pollutants in river water depend on the land use of catchment

areas near these rivers. It has been shown that water in urban streams contains more copper and zinc than in the rural streams (Williamson 1991 c.f. Steven 1999).

The pollutants may have a harmful effect on coastal flora and fauna along their path and their final destination in coastal waters (Kennish 1997). To be in a position to take protective measures against pollution, it is important to monitor the pollutants flowing from surrounding coastal areas and to analyze their possible sources.

### **1.1.1 Effects of stormwater on marine pollution**

Rainwater that flows over impervious surfaces such as roofs, roads, and concrete surfaces is referred to as stormwater (Patin 1982). The stormwater acts as a major nonpoint source of heavy metals in estuarine and coastal water (Forstner and Wittmann 1979). Various types of contaminants from inland areas are transported directly or indirectly to coastal waters in stormwater. Stormwater collected by surface or underground drainage systems contains heavy metals in various concentrations. Copper and zinc are found in high concentrations in stormwater runoff in urban and industrialised areas (Gaw 1997; Kushel and Timperley 1999).

Some modern cities have artificial drainage systems which discharge stormwater into the sea. The discharge of stormwater through artificial drainage systems amplifies the degree of pollution because delivery to coastal areas is more rapid and there is not enough time for the suspended contaminants to settle (Snelder and Trueman 1995). In Auckland, high amounts of copper and zinc were found in the stormwater collected from highly urbanised areas (Timperley 2000).

Both the contaminant generating rate and concentration of contaminants in stormwater vary with the type of land use of the area (Glasby, Stoffers *et al.* 1988; Steven 1999). These contaminants may include (Kennish, 1997):

- Organic nutrients, mainly in the forms of nitrogen and phosphorus

- Heavy metals associated with sewage effluent and stormwater
- Organochlorine compounds from various pesticides
- Industrial wastes and petroleum hydrocarbons

### **1.1.2 Effects of atmospheric air on marine pollution**

Contaminated atmospheric air indirectly facilitates coastal pollution (Clark 1997). Pollutants in the air derived from vehicles and industrial emissions contribute greatly to the chemical pollution of the atmospheric air (Clark 1997). These chemicals in the atmosphere are washed by rainwater and are deposited on land, in water bodies and ultimately end up in coastal waters (Kuschel 1999).

### **1.1.3 Effects of dumping of wastes on marine habitats**

Although it is well known that dumping of untreated wastes directly into waterways can damage aquatic organisms, the practice is continuously performed around the world (Clark 1997). These domestic and industrial wastes ultimately reach the coastal waters causing pollution of the marine environment. The wastes, which lead to marine pollution, have been grouped into six major categories (Gorman 1952),

1. Sewage
2. Marine debris
3. Toxic chemicals
4. Heavy metals
5. Fossil fuels
6. Radioactive materials.

## **1.2 Heavy metal pollution in marine environments**

Pollutants in the aquatic environment, which are not degradable by biological or chemical processes, tend to accumulate in high concentrations in water and

sediments of aquatic habitats (Clark, 1997). Heavy metals are one category of non-degradable pollutants in the aquatic environment and occur both in sediments and water (Clark, 1997). Large amounts of these heavy metal pollutants enter the marine environment through estuaries (Clark, 1997).

The term heavy metal is used synonymously with trace metals and includes both essential and non-essential metal elements. Heavy metals are defined chemically as a group of elements with atomic weights ranging from 63.54 to 200.590, which have similar electronic distributions in the outermost shell of their atoms (Connell, *et.al.*, 1984). The specific gravity of heavy metals is greater than 4.0 (Connell, *et.al.*, 1984). Heavy metals are more readily bioavailable in aquatic environments than in terrestrial environments (Clark, 1997).

Common heavy metals found in aquatic environments are copper, zinc, cadmium, mercury and lead (Kennish 1992). Heavy metals, which are found in aquatic environments, are categorised into two main groups (Viarengo 1985). One group includes iron, magnesium, manganese, cobalt, zinc and copper, which are essential for the normal biochemical functions of animals. Heavy metals of the second group have no known important function in the metabolic pathways in aquatic animals (Viarengo 1985). Elevated heavy metal concentrations in aquatic systems are often toxic to animals living in those environments. The toxic effects of heavy metals on marine organisms depend on various factors. Some heavy metal elements are relatively more toxic than others (Ketchum 1980; Manahan 1993). For example, the relative toxicity of cadmium, lead, copper and zinc have been given in the order of high toxicity to low toxicity as cadmium > lead > copper > zinc (Ketchum 1980).

### **1.2.1 Heavy metal pollution in coastal environments**

A large portion of marine life occurs in habitats in the river water - seawater interphase zones, such as river mouths, estuaries, and the coastal environment

(Klein 1994). Hence, these habitats are important in determining the biological structure of the world's oceans (Klein 1994).

Monitoring for heavy metals will help to develop an understanding of the adverse effects of heavy metals on coastal organisms. Many studies have been conducted to measure heavy metal contaminations in estuarine and other coastal environments (Kennish 1997; Shulkin, Presley *et al.* 2003). This is due to the fact that fresh water encounters a boundary with the sea, which facilitates settlement of pollutants. Also, the change in salinity causes flocculating of suspended sediments, which may contain heavy metals. Under certain conditions increased solubility and mobility of naturally occurring heavy metals in soil cause high concentration of heavy metals in estuarine water (Butler and Timperley 1996). This phenomenon can be amplified by the dumping of the sewage and sludge on the catchment areas of the rivers. A large portion of seafood is harvested from the coastal areas. Therefore, coastal pollution has a significant economical and social impact on human society (Klein 1994).

Heavy metals transported by river waters to coastal environments accumulate in high concentrations in oceanic environments, where they are present in particulate and dissolved forms (Kennish, 1997). As mentioned earlier, polluted inflowing water masses such as river water carry heavy loads of contaminants to the coastal environment. Elevated heavy metal levels in estuarine water due to industrialization and urbanisation are accumulated in aquatic organisms (Jones, Mercurio *et al.* 2000). High levels of metal contents in the tissues of the aquatic organisms due to human activities are currently a growing environmental problem all over the world (Jones, Mercurio *et al.* 2000).

In recent years, there has been a growing awareness of the need to improve the ability to detect and assess adverse effects of contaminants in marine biota. Environmentalists are conducting various studies to monitor and control metal contamination in coastal environments around the globe (Shulkin, Presley *et al.* 2003).



### **1.2.2 Heavy metal pollution in New Zealand coastal environments**

A large number of estuarine systems in New Zealand are moderately or severely polluted by heavy metals (Penny 1984). The most widespread and major source of heavy metals in urban estuaries, in New Zealand is urban runoff (Penny 1984). Large amounts of copper and zinc were detected in urban stormwater run-off in Auckland by Kuschel and Timperley in 1999 (Kushel and Timperley 1999). Taylor (2002), suggested that higher alkalinity in wastewater in Auckland is probably due to the presence of heavy metals in the wastewater. Roberts (2002), has observed high concentrations of heavy metals in the stream water in 12 streams in the North Shore City of Auckland. Two of these rivers, Awaruku and Wairau were included in the present study. Butler and Timperley (1996), reported the presence of cadmium in higher concentrations in Mahurangi river habitats. The heavy metals present in all these freshwater outlets amplify the heavy metal pollution of the coastal environments.

### **1.2.3 Sources of heavy metal pollution in coastal environments**

Some heavy metals enter the marine environment by natural processes, such as gaseous state or aerosols, and may reach the sea surface by dry deposition, precipitation, or by gaseous exchange (Kennish 1997). Hydrothermal activity in deep seawater is another natural source of heavy metals, particularly arsenic and mercury, in the marine environment (Bruland c.f. Kennish, 1992). Amount of heavy metals such as copper, lead and zinc are normal constituents of marine and estuarine environments (Penny 1984). In coastal regions, heavy metals are normally supplied to the sea by river water or as windborne materials following the weathering of soil (Penny 1984). However, high concentrations of heavy metals in coastal environment are mostly due to anthropogenic effects (Nriagu 1989).

Motor vehicles are widespread source of copper, lead and zinc in urban runoff (Kahraman, Olmez *et al.* 1976). The most common sources of cadmium entering the aquatic environment are industrial effluents and sewage (Butler and

Timperley 1996). In general, as the soil becomes more acidic, cadmium becomes increasingly mobile (Butler and Timperley 1996). Therefore, effluents with cadmium placed on land, by the land disposal of sewage and sludge, could easily leak into streams and estuaries (Butler and Timperley 1996). However, it has been found that the Mahurangi catchment consists of extensively farmed areas for livestock production, the sediments and animal tissues of the estuary have significantly high concentration of cadmium (Butler and Timperley 1996). Phosphoric fertilisers which are used in pastureland around Mahurangi estuary contain cadmium greater than 10 mg/kg (Butler and Timperley 1996), and could be the source of high cadmium concentration of these habitats. These higher levels of heavy metals in coastal habitats create undesirable environments, for coastal flora and fauna (Olson and Burgess 1967). Long-term monitoring of copper in Chalk Point Stream in Maryland, revealed the effect of land use of river catchment on the copper concentration in coastal waters (Phillips 1976). Chalk Point Stream Electric station in Maryland, located 20 nautical miles away from the river mouth, used copper tubes for cooling (Phillips 1976). As a consequence they have thoroughly examined copper entering in to the coastal environment (Phillips 1976). It has been calculated that the annual copper loading was 1150 kg in 1982. With the replacement of copper tubes to titanium alloy tubes during 1986 to 1987 the copper content of oysters down stream had decreased significantly (Phillips 1976).

### **1.3 Effects of heavy metals on animals**

Even low concentrations of heavy metal levels in the environment can have harmful effects due to the accumulation of these metals within the body tissues of the aquatic animals. Animals, such as bivalves, can absorb heavy metals either directly from the water or indirectly from their food. Biomagnification is another problem induced by contaminants within the body tissues of animals along the food chain. Biomagnification is the process by which contaminants are accumulated in higher concentrations in animals higher up in the food chain because they receive contaminants from all the animals lower down the food chain (Mance 1987). As a result, humans and other predators who belong to the

higher levels of the food chain may have more heavy metal pollutants in their body tissues than the animals at lower levels (Mance 1987).

Several studies revealed that heavy metal pollutants affect the biology and ecology of the aquatic environment (Ketchum, 1980; Shulkin, Presley *et al.*, 2003). The ecological effects of heavy metal pollution on aquatic organisms depends on the type of pollutant, the concentration and the duration of exposure to the pollutant (Wood 1974). Toxicity can change the community structure and population of organism (Kennish 1992). Also, toxicity can be expressed in organismic, cellular or sub-cellular levels (Kennish 1992).

The biological effects of heavy metals, such as various pathological conditions on aquatic animals, depend on the toxicity of the heavy metal compounds. Kennish (1997) showed that the chemical nature of heavy metal compounds is more important in the determination of the toxicity of heavy metals than the total concentration or the exposure time of the animal to the heavy metal compound. However, animals living in environments contaminated with heavy metals can often regulate metal contents in their bodies within a limited range. Excess heavy metals ingested by animals are excreted, detoxified or accumulated inside the animal bodies. All heavy metal contaminants have harmful effects on marine animals above a threshold concentration in water (Kennish, 1997). The exposure of marine animals to higher levels than the threshold heavy metal concentration results in various pathological and physiological responses, such as tissue inflammation and degeneration, genetic derangement and growth retardation (Kennish 1997).

### **1.3.1 Effects of heavy metals on human health**

Coastal pollution has a potential health risk for humans because people all over the world use coastal organisms as food sources and coastal water for various recreational purposes (Edwards, Edyvane *et al.* 2001). However, most current health risks are associated with consumption of seafood (Han, Jeng *et al.* 1998).

Organic and inorganic pollutants accumulate in the tissues of bivalves, because they have the ability to concentrate toxins by continuously filter feeding on the minute particles in the water. When people consume large amounts of contaminated seafood, they may receive elevated levels of heavy metals in their body organs.

A number of food poisoning cases, due to heavy metal contamination of the coastal environment have been reported from all over the world (Phillips and Rainbow 1992). Countries, such as New Zealand, have introduced legislative protections, such as New Zealand Food Safety Authority (2002), to prevent heavy metal poisoning due to consumption of contaminated sea food.

Different heavy metals have different effects on human health. For example, elements such as cadmium, lead and mercury are more harmful than the other heavy metal compounds (Manahan 1993). Mercury poisoning was the first case of heavy metal contamination reported from seafood. It was reported in Minimata Bay, in the eastern Shiranui sea in 1953, where fish and shellfish were contaminated with mercury (Phillips and Rainbow 1992). Consequently, mercury poisoning due to aquatic contamination has been reported from several other parts of the world, including Sweden, Canada and the USA (Phillips and Rainbow 1992).

Cadmium contamination of the marine environment occurs mainly due to industries, such as electroplating industry, pigments and plastic stabilisation, batteries manufacturing, and industries involving metallic alloys (Muhammad 1992). Cadmium is probably the most biotoxic element in the aquatic habitats (Muhammad 1992). When excess cadmium is absorbed it tends to accumulate in the liver and in the kidneys of the human body (Abbe, Riedel *et al.* 2000). Cadmium has a carcinogenic effect and is an acute toxic hazard (Abbe, Riedel *et al.* 2000). Calcium of the bones can be replaced by cadmium causing brittleness of the bones, and it has also the ability to raise the blood pressure of humans (Gorman 1993). A cadmium toxicity reported in Japan in 1947 caused painful skeletal deformities and the disease was called as itai-itai (Mance 1987).

Copper contamination of the environment occurs mainly due to electroplating industries and sewage effluents (Hickey 1992). Poisoning of copper can lead to stunted growth of humans (Gorman 1993). Long-term over-exposure to copper may cause cirrhosis of the liver and jaundice in humans (Gorman 1993).

Motor vehicle emissions and used automobile batteries act as major sources of lead into the environment (Bunce 1994). Symptoms of excess lead include delayed development in children and disorders of the nervous system leading to mental retardation (Gorman 1993; Edwards, Edyvane *et al.* 2001).

Zinc is naturally present in relatively high amounts in marine sediments (Klein 1994). Zinc used as sacrificial anodes on boats, road runoff, metal finishing industry, battery recycling plants, galvanised iron roofing sheets and paint and dye manufacturing industries are largely responsible for release large amounts of zinc into the coastal environment (Hickey 1992; Kushel and Timperley 1999).

#### **1.4 Importance of the management of the coastal environment**

As described earlier, the contamination of the coastal environment may lead to potential risks to human health and various other adverse effects to coastal organisms. Coastal pollution affects human health mainly through the consumption of contaminated food (Edwards, Edyvane *et al.* 2001). Potential health risks on humans can be prevented by developing a monitoring system through which potential impacts of marine pollutants can be detected long before they become a risk to human health (Edwards, Edyvane *et al.* 2001). Early detection of unfavourable changes occurring in the aquatic habitats allows authorities to take precautions towards protecting the coastal habitats. Management through monitoring of the coastal environment allows the authorities to plan future industries and land use to minimise the possibilities of pollution in aquatic habitats. Knowledge of the relative abundance of heavy metals in river mouths helps the governing bodies to implement conservation

plans to protect the ecology of the local biota. Environmental management strategies, such as the landuse hydraulic interaction (LHI) introduced by the National Institute of Water and Atmospheric Research (NIWA), aimed to protect urban streams by managing stormwater flows (Kushel and Timperley 1999), are effective in the protection of coastal waters.

Monitoring programs such as “Mussel Watch” are currently being carried out to identify the levels and effects of pollutants in marine habitats (Goldberg and Bertine 2000). The prevention of marine pollution could be achieved by improving the quality of coastal water. Researchers all over the world are monitoring wastewater discharges, controlling and eliminating sources of land-based pollution in order to protect marine habitats.

## **1.5 Monitoring of coastal environment**

The assessment of various ecological, biological, chemical and physical parameters of coastal habitats is widely used to monitor the coastal environment (Phillips 1977).

### **1.5.1 Ecological monitoring of the coastal environment**

Ecological survey procedures involve population counts of organisms and observing the changes in the abundance and diversity of species comprising communities, associated with specific contaminated environments (Phillips 1977; Samoiloff 1989). Organisms used in ecological monitoring in aquatic habitats have the ability to indicate the concentration and types of pollutants. Ecological monitoring includes observation of decreasing or increasing of the number or complete disappearance of the organism in the environment. Therefore, ecological monitoring methods help to identify the critical factors affecting the organism (Soule and Kleppel 1987; Jamil 2001). Ecological survey procedures are useful mainly for the qualitative assessment of the environment. The main limitation of this method is that the population levels

are dependent on a wide variety of ecological factors other than contaminant types and concentrations (Samoiloff 1989).

### **1.5.2 Biological monitoring of the coastal environment**

According to Samoiloff (1989), the environment can be assessed biologically by the biomonitoring of morphological abnormalities in individuals and populations. The biological analysis of the environment involves using ratios between highly sensitive species and resistant species (Samoiloff 1989). This approach requires base line data of the relative size of the resistant species in order to measure contamination of the environment (Samoiloff 1989). However, it is difficult to have an accurate assessment of the environment using this method due to lack of acceptable baseline data (Samoiloff 1989). There is no universally accepted available baseline data because the overall sensitivity and resistance is highly influenced by local population differences and the local environmental variations (Samoiloff 1989). Another disadvantage of this method is that it provides information only on the adverse effects of contaminants (Samoiloff 1989).

### **1.5.3 Monitoring the coastal environment using chemical methods**

Chemical analysis of water, sediments, plant or animal tissues are widely used to detect the impact of anthropogenic contaminants in marine and estuarine environments (Phillips 1977). However, some limitations have been observed in some of these analytical methods (Phillips 1977).

#### **1.5.3.1 Chemical analysis of water**

One of the problems of measuring heavy metal concentration in water samples is that the concentrations may be too low to detect (Phillips 1977; O'Connor 1998). Measuring these very low amounts in water requires pre-concentrating processes (Rainbow 1995).

The other problems associated with detecting heavy metals in water include, that the concentration vary very rapidly due to environmental factors such as seasonal changes, time of the day, amount of fresh water runoff (Phillips 1977; Rainbow 1995). Metal concentration in water could also be affected by the depth of sampling, fluctuations of industrial discharges and hydrological factors, such as tides and currents (Phillips 1977; Rainbow 1995). Therefore, even when the metals present in high concentrations in water, the results cannot be used as an index of pollution. The measured heavy metal concentration in water would not represent the biological important fraction of the heavy metals (Rainbow 1995). However, this data provide only a quantitative assessment of the total metals present in the aquatic environment at the time of sampling (Rainbow 1995).

#### **1.5.3.2 Chemical analysis of sediments**

As an alternative method to monitor the heavy metal in aquatic environments, the chemical analysis of sediments has some advantages over that of water (O'Connor 1998). Heavy metals adsorbed onto suspended organic and inorganic particles can accumulate in aquatic habitats, and their concentration can be reflected in sediment analysis. Metal adsorption of sediments is a surface effect (Frazier 1976; Soares, Boaventura *et al.* 1999; Huang and Lin 2003). This phenomenon of metal adsorption on to the surface of sediment is mainly determined by physical and chemical characteristic of the surface. Both organic and inorganic ion composition in sediments influence the metal adsorption onto it (Luoma 1989). When the organic component of the sediment increases, its metal content will increase in a linear fashion (van Roon 1999). The biological oxidation of organic carbon changes the form of iron, manganese and sulphur in sediment which in turn increase the binding of trace elements to sediments (van Roon 1999). Correlation between iron and trace metal concentration in sediment has been shown by Presley, *et al.* (1992). Surface area, which is inversely related to the particle size, has a greater influence on the metal adsorption. In addition to particle size, heavy metal measurements



obtained by sediment analysis depend on the rate of particle sedimentation and rate of heavy metal deposition (Phillips 1977). The concentration of heavy metals in sediment depends on the ratio of quantity of metal deposited on the sediment particles to sediment deposited over a given period of time (Phillips 1977). Sampling time and location of sampling may also have an influence on the final results (Phillips 1977). However, the major drawback of this method is that measured heavy metal level may not accurately represent its concentration in the aquatic environment. The reason being the ability of the metals to adsorb on to the metal particles depends on the type of metals and the particulate content and its nature (Phillips 1977). The heavy metal content in sediments is not directly related to the bioavailable fraction of the environment. Because only a certain amount of heavy metals present in sediments can be absorbed by aquatic organisms. The fraction of heavy metals in sediments that can be absorbed by aquatic organisms is known as the bioavailable fraction. Thus, the heavy metals in sediments do not represent the biologically important heavy metal content of the environment (Phillips 1977).

#### **1.5.3.3 Chemical analysis of animal tissues**

The universally acceptable biological approach to monitoring environmental pollution is the chemical analysis of body tissues of organisms to detect the level of contaminants in the environment (Samoiloff 1989). The analysis of oyster and mussel tissues has the advantage that they are able to concentrate heavy metals to analysable levels even when the environmental metal concentration are very low, such as parts per trillion or parts per quadrillion (Goldberg and Bertine 2000). Studies conducted in coastal areas proved that coastal organisms have the ability to accumulate heavy metals, such as mercury and cadmium, up to high levels even when those metals are in hardly detectable concentrations in water (Penny 1984). The chemical analyses of plant and animal tissues to measure heavy metal concentration in aquatic habitats can be used to avoid limitations in water and sediment analysis (Phillips 1977). Bioaccumulated heavy metals can be measured either using whole animal or individual tissue samples on a dry or wet weight basis (Fisher and Reinfelder

1995). This approach provides a measurement of contaminant levels, as well as, the real effects of contaminants on organisms either singularly or cumulatively (Thomas 1975; Samoiloff 1989). More precise values can be obtained for heavy metal concentrations in aquatic environment using plants or animals, because tissues of these organisms are not subject to contamination during the process of analysis, and provide time integrated measures of metal contaminations (Rainbow 1995).

Changes in heavy metal concentration in these organisms indicate changes in pollution levels in the environment (Phillips and Rainbow 1992). These types of organisms, known as biomonitors, can be used to monitor the environmental factors over time (Phillips and Rainbow 1992). The greatest advantage of using animals to monitor the environment is that the measured concentration of contaminants are directly related to the bioavailability of contaminants (Phillips and Rainbow 1992). Using organisms to analyse contaminants, such as heavy metals, provides a direct assessment, more comprehensive and realistic measurement of the bioavailability in the aquatic environment (Samoiloff 1989; Silva, Rainbow et al. 2001).

However, the disadvantage of using organisms to monitor the environment is that they cannot indicate precisely which environmental factor or condition is responsible for the contamination (Lenihan and Fletcher 1978).

## **1.6 Bioaccumulation of heavy metals in coastal animals**

The accumulation of the absorbed heavy metals, within the body tissues of animals, as an element or compound to concentrations greater than that found in its environment are referred to as bioaccumulation (van Roon 1999).

Heavy metals in the aquatic environment are absorbed by bivalves either directly through the gills in the process of respiration or indirectly with food particles (Clark 1997). Bivalves are able to absorb more heavy metals through gills than their gut (Tinsley 1979). During the process of respiration large volumes of water pass through the gills of bivalves and the large surface area of the gills facilitates the absorption of heavy metals (Tinsley 1979). Toxic substances may tend to accumulate within the animal tissues along the food chain. Tinsley (1979) has shown that bivalves are primary consumers, and therefore the chances of accumulating of heavy metals through the food chain are very remote. However, Phillips (1977) suggests that uptake from food is the most important route of entry, as dissolved concentration of heavy metals in water is very low.

Bivalves have a limited ability to metabolise and depurate the heavy metal pollutants they absorb from water and sediments (Kim, Powell *et al.* 2001). However, various physiological mechanisms are triggered by bivalves, such as oysters, to expel absorbed heavy metals in order to control the heavy metal content in their body tissues (Butler and Timperley 1996). By these mechanisms heavy metals are neutralised within the amoebocytes of the oysters (Butler and Timperley 1996). It has been observed that the species *Crassostrea gigas* expels half of the ingested cadmium contaminants within 23 – 60 days after ingestion as a physiological controlling measure (Butler and Timperley 1996).

### **1.6.1 Quantifying the bioaccumulation**

Different bivalve species have different capacities to accumulate heavy metals within their body tissues. The capacity to accumulate heavy metals in animal tissues is measured by a concentration factor. This is given as a ratio of trace metals in animal to trace metals in sea water (Huanxin, Lejun *et al.* 2000).

The calculated value of the concentration factor (CF), of copper for oysters is 7500. High values of CF for copper in oysters indicate that large amounts of copper have been accumulated within oyster tissues (Clark 1997). Due to

practical limitations of analysis of the heavy metal content in the sea water at the sampling sites, this method of calculating the accumulation of heavy metals in tissues has not been adopted in this study.

### **1.6.2 Factors influencing the bioaccumulation of heavy metals in animals**

Rates of bio-accumulation vary with taxonomic species such as oysters and mussels (George 1983a; van Roon 1999). In the case of bivalves, physiological processes of the organism, as well as, environmental factors influence the rate of bioaccumulation (Okazaki and Panietz 1981; Shulkin, Presley *et al.* 2003).

Bivalves, like other organisms have complex interactions with the environment. Variations of bioaccumulated heavy metals within body tissues of bivalves are a result of a complex combination of biotic and abiotic factors of the environment (Technical Publication 2001). Abiotic factors such as the physical and chemical properties of the environment and the chemical nature of the heavy metals influence on the heavy metal accumulation of bivalves (van Roon 1999).

Temperature and salinity are important physical factors that affect bioaccumulation of bivalves. Relatively high temperatures in seawater promote both the cadmium and copper absorption in the bivalves (Abbe, Riedel *et al.* 2000). It has been shown that temperature plays an important role in cadmium uptake in *Mytilus edulis* (Jackim, Morrison *et al.* 1977; Zaroogian 1980). Zaroogian (1980) observed a significant positive relationship between the cadmium concentration and the temperature of the seawater, and the rate of cadmium accumulation in the oyster species, *Crassostrea virginica*. Studies carried out during summer by various authors have shown that temperature has an effect on cadmium uptake by bivalves (Frazier 1976; Jackim, Morrison *et al.* 1977; Phillips 1977). The same authors have shown that temperature has no significant influence on cadmium uptake by bivalves in winter months. Low temperatures during the winter months may have little effect on cadmium uptake. Comparatively high water temperatures prevailing during summer may have more significant effect on cadmium uptake. The likely cause for the

decreased concentration of heavy metals in body tissues of the mussels living in environments with low temperatures may be due to the reduced pumping activity through their gills at these low temperatures (Zaroogian 1980).

The rate of absorption and accumulation of cadmium and lead by oyster species *Saccostrea echinata* has been favoured by low salinities such as, 20 ‰ than the accumulation rate in higher salinities such as, 36 ‰ (Denton and Burdon-Jones 1981). However Phillips (1976), observed decrease of net uptake of lead in mussel species *Mytilus edulis* at low salinities (Phillips 1976). The salinity effect on lead uptake would cause an under estimation of lead concentration in bivalve tissues in areas where the salinities are lower than the normal values (Phillips 1976). However, absorption and accumulation of zinc in the tissues of mussels are affected neither by the temperature nor by the salinity of the seawater (Phillips 1976).

Depth of the water column is another physical factor that may affect the bioaccumulation in bivalves. It has been shown that oysters can concentrate zinc up to 50 times more than the concentration found in bottom sediments (Rebelo, Amaral *et al.* 2003). Some authors suggest that this accumulation rate may be due to slow or inefficient depuration mechanisms (Rebelo, Amaral *et al.* 2003).

Metal concentrations in the soft tissues of oysters may be influenced by the seasonal changes in the environment (Galtsoff 1964, Osuna, Espericueta *et al.* 1995). This may be due to seasonal changes in their biological cycles or seasonal changes in the availability of metals in their environments.

Levels of heavy metal concentrations in soft tissues of oysters vary with rainfall. However, there is no evidence for uniform relationship between rainfall and metal concentration (Rebelo, Amaral *et al.* 2003).

The chemical nature of heavy metals in aquatic environments plays an important role in bioaccumulation in bivalves. Heavy metals are mainly present

in dissolved or suspended forms as free ions or as complex compounds in aquatic habitats. It is the free ions than the water soluble fraction which is bioavailable and ecologically more reactive (Kennish 1997). A higher rate of absorption of the free ion than the complex compounds of heavy metals was observed in oysters by Cunningham and Trip (1973). They found ionic mercury (as  $\text{HgCl}_2$ ) was absorbed at four times the rate of organic mercury in *Crassostrea virginica* in the same environment (Cunningham and Tripp 1973).

Biological factors such as reproductive cycle, age and body size of bivalve species exert an important influence on the accumulation of trace metals (Boyden 1974). The study carried out by Paez-osuna *et al*, (1995) showed that copper, cadmium and zinc concentrations are at their maximum during resting or spent stages of gonad development and a gradual drop was observed with the development of gonads. Similar to cadmium and zinc, reduced lead concentrations in oyster tissues has been observed during the spawning period of the life cycle (Zaroogian, Morrison *et al*. 1979). This probably is due to temporary loss of the oyster's reduced capacity to retain or accumulate lead during the spawning period. During the post spawning stage their metal concentrations increases again, perhaps oysters may not resume accumulation of lead until they had completed post-spawning metabolic adjustments (Zaroogian, Morrison *et al*. 1979).

In the case of manganese, there has been a possible correlation associated with the gonadal tissues. The gonadal development and biochemical variation associated with reproduction, rather than body size are more important in the bioaccumulation of manganese (Galtsoff 1964). It could be argued that the effect of temperature on metal up-take by bivalves is related to increased physiological and metabolic processes. It is generally assumed that animals can increase the metal absorption with the increase of temperature within a normal metabolic range. Therefore metal uptake and its accumulation by bivalves in temperate regions are greatly influenced by temperature (van Roon 1999). However, the study conducted by Denton (1981) has shown that increased metal uptake with temperature is not simply related to the metabolic activities such as flow of water through the mantle cavity, because the effect is more pronounced

for mercury and cadmium than for lead. This suggests that the mechanism involved in lead accumulation from sea water is different and less affected by temperature than those of mercury and cadmium. It is clear that the net uptake of metal by bivalves is not due to a single factor, but due to complex interaction of a number of biological and environmental factors. The seasonal fluctuations of trace metal concentrations with the exception of manganese were reciprocal to the seasonal changes in tissue weights of the individual animal. It could therefore be justified that total metal content of each individual changed little throughout the year. Weight changes were in turn related to the sexual cycle, with a minimum in spent stage considered with the seasonal maximum of trace metal concentration (Zaroogian 1980). Care should be exercised when interpreting the above bioaccumulated metal concentrations with respect to low chronic or short term concentrations.

### **1.6.3 Effects of Bioaccumulation in bivalves**

As bivalves are sessile organisms they have various mechanisms to protect themselves from toxic effects of heavy metals. Some bivalves have the ability to neutralize the toxicity of heavy metals and to store toxic materials at cellular levels of the body tissues (Frazier 1976; Viarengo 1985). However, a number of adverse effects of heavy metals on the health and productivity of bivalves have been reported in the literature (Krogh and Scanes 1996). The study carried out using two oyster groups; a control and a group exposed to heavy metal contaminants, by Frazier (1976) found that average shell thickness ( $\text{mg shells/cm}^2$ ) was 16% less in exposed oysters than controls. However, the growth of the two populations based on two indices, weight of soft tissues and cell dimension, were identical (Frazier 1976). A possible hypothesis explaining this effect is that metal contamination of oyster soft tissues interfered with the calcification process. The basis of this hypothesis is that several metals, including copper and cadmium have been shown to inhibit zinc metalloenzymes (Viarengo, 1985), in particular alkaline phosphatases and carbonic anhydrase, which are involved in shell metabolism (Viarengo, 1985). Since the concentrations of both of these metals were elevated in soft tissues of exposed

animals above the control group it could be argued that the metal toxicity may have been involved in shell thinning. The reduction in shell thickness is an example of sub-lethal responses of an organism to environmental stress. This response can have a significant impact on the ecological distribution of the organism, not as a result of direct mortality, but by rendering oysters more susceptible to predation by oyster drills, boring sponges and blue crab (Frazier 1976).

Some heavy metal compounds, such as tributyl tin oxide (TBT) affect shellfish growth even in very low concentrations ( $0.01 \mu\text{g l}^{-1}$ ). TBT causes thickening of the shell of pacific oyster, *Crassostrea gigas*, and the same chemical greatly reduces the size of this oyster species (Clark 1997). The physiology, reproduction and development of the bivalves can be affected by sub-lethal levels of heavy metal toxicity in the environment (van Roon 1999). For example, morphological changes, such as the greening and thinning of shells, or retardation of growth have been observed in oysters when they were exposed to sub-lethal levels of heavy metal concentrations in the surrounding environment (Nielsen and Nathan 1975).

Bivalves show adaptive biochemical processes to reduce the metal toxicity. Metallothioneins, low molecular weight proteins with high affinity for heavy metal cations are widely distributed in animals (Roesejadi 1980; Viarengo 1985). Metallothionein usually are not saturated by a single metal, but 6-7 ions of copper, zinc and cadmium, mercury and silver, when they coexist. When cadmium, mercury or excess copper enters the cell, these metals are able to displace zinc from zinc copper thionein normally present in cytosol. This buffer effect of pre-existing thionein could represent the first step in the process of heavy metal homeostasis. If the concentrations of the metal entering into the cell exceed the saturation point of physiological pool of zinc copper thionein excess cations have been shown to stimulate the synthesis of new thionein at the nuclear level or at the ribosomal level. Although the metal concentration in the cell may be abnormally high, most of it present in non toxic form bound to neo-synthesized thionein. It could therefore, be assumed that only if the rate of



influx of metal into the cell exceed the rate of metallothionein synthesis and / or if the maximum value of the thionein produced in the cell is exceeded by the metals, which can then interact with cellular components to cause toxicity (Viarengo 1985).

Even though adult oysters can accumulate high amounts of copper and zinc without adverse effects to their biology (Roper, Pridmore *et al.* 1991), larvae are very sensitive to copper and zinc concentrations in the environment (Clark, 1997). Mance (1987), observed abnormal developments in the adult and larvae of *Crassostrea gigas* when they were exposed to higher concentrations of cadmium, copper, lead and zinc heavy metals under experimental conditions. A development of thin watery translucent tissues and abnormal shells were observed in *Crassostrea gigas* when they were exposed to contaminated environments (Okazaki and Panietz 1981).

### **1.7 Condition Index of oysters**

Condition index (CI) is a measurement used to quantify the condition of bivalves both in scientific research and commercial purposes, and it represents the amount of living tissues in relation to the amount of the shell of the organism (Davenport and Chen 1987). CI is used to assess the “health” or “fatness” of bivalves (Roper, Pridmore *et al.* 1991), and can be calculated either for an individual or for a whole population (Gosling 2003). Several biochemical and physical parameters have been described by Davenport and Chen (1987) to assess the CI of bivalves. Physical condition of bivalves can be determined using wet flesh weight, dry flesh weight, shell weight and shell volume. According to Davenport and Chen (1987) the biochemical condition of bivalves can be assessed using protein and glycogen content of the tissues of the organism (Pridmore and Roper *et al.* (1990).

### 1.7.1 Measurement of CI

Davenport suggested that more accurate values could be obtained for the CI of the oysters by using one of the following formulas;(Davenport and Chen 1987)

$$CI = \frac{\text{Wet meat weight} \times 100}{(\text{Total volume} - \text{shell volume})}$$

$$CI = \frac{\text{Dry meat weight} \times 100}{\text{Shell weight}}$$

CI calculated using the shell volume of oysters is less precise because shell volume depends on the thickness of the oyster shell (Brown and Hartwick 1988). Shell thickening of the oysters due to environmental pollution (Pridmore, Roper *et al.* 1990) could give false values for CI when using dry weight / shell volume method. During sampling of oysters, visual examination confirmed that oysters sampled at the river mouth of Wairau had thicker shells than the oysters sampled at the other river mouths. To overcome this problem the CI for this study was calculated using dry weight and shell weight measurements. This same method that was used to calculate the CI of the oysters in the Manukau harbour, in New Zealand (Pridmore, Roper *et al.* 1990). The method used to calculate the physical condition of the oysters, is based on the assumption that the tissue weight of the oysters varies according to the growth of the animals. Hence the growth of the oyster tissues has directly influence on the condition of the oysters.

### 1.7.2 Factors affecting condition

The major factors affecting the condition of bivalves depend on the health and growth of the organisms. Toxicity resulting from heavy metals can adversely affect the growth and other metabolic functions of individual organism or the whole populations of bivalves in a particular habitat. Therefore, heavy metal concentration of the tissues of bivalves can affect the condition of bivalves. Seasonal fluctuations of physical factors (*i.e.*, temperature, salinity and particulate matter), chemical factors (*i.e.*, concentration of heavy metals and

organic compounds), and biological factors (*i.e.* bacterial population, the abundance of phytoplankton in water and reproductive cycle) are some of the main factors influencing on the condition of bivalves (Pridmore, Roper *et al.* 1990; Roper, Pridmore *et al.* 1991; Hickey 1992). Various chemical pollutants reduce the condition of bivalves. Hickey (1992), has described pollution related differences of condition in bivalves. According to that study low growth rates and condition of bivalve species *Perna canaliculus* has been observed in environments with a variety of pollutants including heavy metals. A relationship between the condition and the chemical pollutants in water was observed by Pridmore, Roper *et al.*, (1990) in the Manukau Harbour. The same authors found a reduction in the condition due to the presence of organic compounds such as DDT and Chlorophenols. Bender, Hargis *et al.*, (1988) showed concentrations of about 20µg (g/dry wt) of Polynuclear Aromatics Hydrocarbons (PAH) in tissues can lower the condition of oysters.

Availability of food is another factor which affects on the condition of bivalves. Roper *et al.*, (1991) suggest that higher total particulate matter (TPM) concentration in water can lower the condition of the organism. Higher concentration of TPM in water may interfere the feeding of bivalves by inhibiting or slowing down the oyster's filtration rate (Rebelo, Amaral *et al.* 2003).

## **1.8 Use of animals as biomonitors to detect the heavy metal concentration in coastal habitats**

As described earlier, a very useful way to study the ecological and biological importance of heavy metal pollution is to measure the bioaccumulated heavy metal concentration in animal tissues. Bioaccumulation in bivalves is strongly influenced by human activities. A study revealed strong statistical correlation between human population density and both lead and tributyl tin (TBT) concentration in mussel tissues of specimens in coastal areas in the US (O'Connor 1998).

As has been pointed out, the physical and chemical conditions of the selected coastal areas are important factors to consider when selecting an organism to monitor the environmental pollution (Phillips 1977). For example, species in the genus *Mytilus* have been used to monitor catchments in temperate regions (Osuna, Espericueta *et al.* 1995). *Crassostrea gigas* is an excellent sentinel organism for marine pollution. Like the genus *Mytilus*, in the tropics *Crassostrea gigas* also is widely distributed in tropical and sub-tropical coasts of the world (Osuna, Espericueta *et al.* 1995). A number of species of gastropods and bivalves have been used as biomonitors in various studies of coastal pollution in New Zealand (Pridmore, Roper *et al.* 1990; Hickey 1992; van Roon 1999). Pridmore, Roper *et al.* (1990) used *Crassostrea gigas*, while van Roon (1999) used gastropod species *Amphibola crenata* as biomonitors for heavy metals. In order to adjust to environmental changes, organisms modify the chemical composition of their body tissues, physical appearance, and their biological activities. Olivier *et al.* (2002), observed changes in the behavioural, physiological and biochemical patterns of bivalves as a response to a heavy metal pollutant in the environment. It is important to have accurate knowledge of the physiology of heavy metal metabolism, feeding, life history, breeding season, length of life, age structure of population of the organism, in order to select good sentinel organism (Rainbow 1995; Shulkin, Presley *et al.* 2003).

The organisms selected for the environmental monitoring purposes should reflected the degree of pollution and types of pollutants in the environment (Rainbow 1995). An ideal organism for environmental monitoring should posses the ability to survive in the laboratories under experimental conditions as well as in natural environments (Soule and Kleppel 1987).

As described earlier, the forms in which heavy metals are available in the aquatic environment are important because the rate and type of heavy metal accumulation in organisms are dependent on the forms of heavy metals they use. For example, suspension feeders, like oysters and mussels are well suited for the monitoring of heavy metals, both in suspended particles and in seawater (Galtsoff 1964). Organisms, which absorb only dissolved sources of heavy

metals, can only be used to monitor the dissolved heavy metal content of the environment (Rainbow 1995).

### **1.8.1 Use of bivalves as biomonitors**

Mussels and oysters are widely used to monitor coastal pollution, because they possess many suitable characteristics; (Phillips 1977; Vernberg, Thurnberg *et al.* 1979).

Some of these characteristics are:

- They are common in areas such as some estuaries and coastal areas.
- Their sessile nature helps to determine the degree of pollution in the particular location where they live.
- Their relatively long life span allows for a long-term effect study of pollution.
- Their broad geographic distribution permits the study of pollution over a widespread area.
- They are readily available because they tend to have high population densities.
- Individuals with known age, genetic percentage and environmental background can be obtained from commercial operations for research purposes.

As oysters and mussels fulfil the requirements of the bio-indicator concept, they are used in sampling studies such as those carried by “Mussel Watch” Programme, in USA (Goldberg and Bertine 2000). A monitoring scheme ‘The Mussel Watch’ was designed and established in 1975, to monitor the status and long term trends of four pollutants in marine waters: artificial radionuclides, petroleum hydrocarbons, chlorinated hydrocarbons, and metals (Goldberg and Bertine 2000). Initially, these pollutants were measured in the east, west and Gulf Coasts of the United States and the Great Lakes (Kim, Powell *et al.* 2001). Later the technique used in “Mussel Watch” was adopted by national and international organisations.

It is important to bear in mind various anomalies of metal accumulation in molluscs when they are used to monitor the environment because bioaccumulation depends on various physiological and behavioural differences of bivalves. For example, bivalves in localities with high amount of suspended food particles filter a small volume of contaminated water to obtain required ingestion level. Thus, lesser amount water filtration allows them to accumulate a lesser amount of metals within their body tissues (Shulkin, Presley *et al.* 2003).

### **1.9 Heavy metal pollutants in sediments**

Sediment is a general term for both suspended and deposited material in the aquatic environment. Samoiloff (1989) indicated that the heavy metals in sediments usually enter the body systems of animals through:

- Contact with sediments.
- Ingestion of sediments by the organism.
- Desorption of contaminants from sediments to water body which is used by the target organism.

The size of the sediment particles may vary from less than 1  $\mu\text{m}$  to coarse sand and gravel of 1 to 2 mm or more in size. Sediments act as a natural absorbent for a number of contaminants, including heavy metals (Samoiloff 1989), and the concentration of heavy metals in sediments is always higher than the concentration of heavy metals in the water (Rainbow 1995). Hence, sediments provide a concentrated pool of metals for analysis in the aquatic environments (Luoma 1989).

### **1.9.1 Relationship of heavy metal content between sediments and the Organisms**

As bivalves live in the water sediment interphases, they have a potential to bioaccumulate heavy metals from contaminated sediments and water (Huanxin, Lejun *et al.* 2000). The heavy metal concentration in bivalve tissues gives a more reliable measurement of heavy metal concentration in sediments than in the water (Shulkin, Presley *et al.* 2003). The concentration of heavy metal contaminants detected by the analysis of bivalve tissues is not directly proportionate to the concentration of the heavy metals in the sediments, but is related only to extractable metal forms in sediments (Shulkin, Presley *et al.* 2003).

### **1.9.2 Factors effecting heavy metal adsorption of sediments**

Concentrations of contaminants in sediments are influenced by grain size and the organic carbon content of sediment particles (Gaw 1997). Sediment grain size is one of the most important factors in determining the concentration of trace elements (Gaw 1997). Grain size is inversely proportional to surface area to which the heavy metal contaminants bind. Fine grain size shows high affinity for heavy metal concentration (Penny 1984; Gaw 1997). Hence, the concentration of surface bound heavy metals is greater in smaller grain size sediments (Penny 1984; Gaw 1997; Burden 2002).

Table 1.10.2 shows an example of the relationship between grain size and concentrations of Copper, Lead and Zinc. These values are obtained from a sediment study conducted in Puhinui Stream in New Zealand, by Gaw in 1997.

**Table 1.1** Relationship between grain size and concentration of Copper, Lead and Zinc (Gaw, 1997).

Particle size range $\mu\text{m}$ .	Copper ( $\mu\text{g/g}$ )	Lead ( $\mu\text{g/g}$ )	Zinc ( $\mu\text{g/g}$ )
<b>1-10</b>	39	78	1067
<b>11 - 30</b>	43	60	623
<b>31 - 60</b>	28	41	479
<b>61 - 150</b>	23	27	308

Particles less than 63  $\mu\text{m}$  accumulate greater concentrations of contaminants than coarse particles (Burden 2002). Shulkin *et al*; (2003) showed that sandy sediments of polluted localities contain more metal contaminants than fine grain sediments from non-polluted localities. The leacheble metal concentration is 100 times greater in sediments at localities near city landfills than background values (Shulkin, Presley *et al.* 2003).

There are factors, other than grain size, that govern heavy metal content in sediments. Gaw (1997) found a positive relationship between the organic matter content and the heavy metal concentration of the sediments. However the organic matter content is not the major factor that control the heavy metal content in the estuarine sediments (Martincic, Kwokal *et al.* 1990).

### **1.9.3 Disadvantages of sediment analyses**

Some heavy metals in the aquatic environment cannot be detected by sediment analysis due to the inconsistent patterns of distribution. Metal contaminants such as aluminium, iron and lead tend to associate with sediment particles rather than exist in the water column in solution. (Phillips and Rainbow 1992), while the contaminants of arsenic, silicon and cadmium tend to exist in dissolved forms (Fresenius, Quentin *et al.* 1988; Phillips and Rainbow 1992). Other elements except the above mentioned are intermediate in action, but depend heavily on chemical and physical factors such as temperature salinity and pH



(Phillips and Rainbow 1992; Gaw 1997). Due to these reasons, the contaminant concentration of sediments does not simply reflect the absolute magnitudes of the contaminant concentration of the sampling point.

### **1.10 Study aim**

This study aimed to investigate the cadmium, copper, lead and zinc concentrations in five river mouths along the north eastern coast of Auckland, New Zealand (Mahurangi, Awaruku, Waiake, Taiorahi and Wairau). The heavy metal concentration and condition index of oysters and sediments at the mouths of the streams were compared to environmental conditions and catchment characteristics.

### **1.11 Summary**

Pollution of the marine environment is a growing problem all over the world. The contaminants reach the marine environment in a number of ways. Heavy metals are one of the main contaminants, which affect coastal fauna and flora. Monitoring of the marine habitats using animals such as oysters is widely used to study the marine and coastal environments. In this study Pacific oysters were used to monitor the shallow coastal areas near to the river mouths of Mahurangi, Awaruku, Waiake and Taiorahi Wairau, situated along the north east coast of Auckland.

## **Chapter 2**

### **2. Materials and methods**

#### **2.1 Study sites**

In this study, the sites were categorised and selected on the basis of available information of activities in the land use of the adjacent catchments (Butler and Timperley 1996; Roberts 2000; Gulliver 2001). The study sites were shallow coastal areas at the mouths of the Mahurangi Awaruku, Waiake, Taiorahi, and Wairau streams in Auckland, New Zealand (Fig. 1). All of these river mouths are situated within 80 km of the north east coast of Auckland. These river mouths opened into a variety of coastal areas, such as recreational beaches (site 1 and 2), sewage pumping station (site 3), boat ramp (site 4) and boat building complex (site 5). Adjacent catchment areas of these river mouths varied from extensive farmlands to heavily urbanised and industrialised land use. The Mahurangi stream runs mainly through undeveloped farmlands, hence, the catchment area consists mainly of pasture lands (Butler and Timperley 1996). The Awaruku stream runs through agricultural lands and through heavily urbanised areas (Gulliver 2001). The Waiake, Taiorahi, and Wairau rivers receive water mainly from the residential areas of Waiake, Murray's Bay and Milford respectively. The catchment area of Wairau stream lies within the North Shore City's primary industrial area (Roberts 2000). The sampled oyster beds were situated along these sites. The densities of the oyster beds are different from site to site. Sampled oyster beds at Awaruku river mouth had a

thin population density compared to other sites. Selected sampling sites at Mahurangi and Wairau were ecologically more significant than the other sites due to the presence of heavy algal growth close to the low tide area of the shore. A boat building site was situated at Wairau estuary. Oysters from Mahurangi creek about 80 km north of central Auckland were taken to measure the baseline concentration.



**Fig.1** Location of study sites at Mahurangi, Awaruku, Waiake, Taiorahi and Wairau, Auckland New Zealand

## **2.2 Sampled organism; Pacific oyster**

The Pacific oyster is also commonly known as Japanese oyster, Miyangi oyster and Giant Pacific oyster. The Pacific oyster, *Crassostrea gigas* is classified as belonging to the Phylum Mollusca, Class Bivalvia, Order Pterioidea, Super family Ostracea. The Pacific oyster; *Crassostrea gigas*, is a native of Japan. The distribution of this species is confined to a broad belt of the intertidal zone of the coastal areas within the latitude 64° N and 44° S in of the world (Geltsoff, 1964). The Pacific oyster was introduced to New Zealand accidentally in the 1960's, and is now well established on the northern coastline of Auckland (Pridmore, Roper *et al.* 1990). This species is now found naturally in the lower mid tidal zone throughout the New Zealand (Saunders and Creese 2000). Pacific oysters live in both polluted and unpolluted environments, can tolerate a wide range of temperature, salinity and particulate matter and have now become a popular commercially farmed oyster in this country (Saunders and Creese 2000). For example Pacific oysters are found in relatively high numbers such as 70- 450 /m<sup>2</sup> on the northern shore of Manukau harbour which receives water contaminated with various types of pollutants from the Auckland metropolitan area (Pridmore, Roper *et al.* 1990).

### **2.2.1 Morphology**

The shell of the Pacific oyster has an irregular shape with ribbed and sharp edges. The top shell has a fairly flat surface and the bottom shell is cupped shaped. Broad shallow valves are chalky white in colour, and there are three longitudinal colour zones of intermittent purple splashes on a yellow white background (Taylor, 2001). Pacific oysters differ from the rock oyster; *Saccostrea cuculata* in that they have layers of frilly plates over their shell (Crow, 1999).



**Fig.2.2** Outer surface view of the top shell and the inner surface view of the bottom shell of *Crassostrea gigas*

### **2.3 Advantages of selecting *Crassostrea gigas* for this study**

Species *Crassostrea gigas* is cosmopolitan and could be collected at the coastal study sites without significantly affecting their population. The oysters exhibit a preference for estuarine habitats, hence sampling could be easily done during the low tide. The species, *Crassostrea gigas* finds the climatic condition of New Zealand coastal habitats favourable to complete its life cycle. During the life cycle of *Crassostrea gigas* in New Zealand coastal waters develop their gonads from early August to February, and spawning occurs from February to April, reaching maximum in April (Peter 1990). The ideal temperature for spawning lies above 15°C and depends on species (Young 1960).

## **2.4 Sampling design**

Oyster and sediment samples were collected at the mouths of the Mahurangi, Awaruku, Waiake, Taiorahi, and Wairau streams. Samples of oysters and sediments were collected, each month between November 2002 and October 2003. The twelve months period was selected to include the four major seasons. Samples of oysters and sediments were usually collected between 15<sup>th</sup> – 20<sup>th</sup> of each month of the sampling period. However, the exact day, on which sampling was carried out depended on the rainfall and the time of the low tide. Sampling was carried out on a day with little or no rainfall. Sampling was done during low tide in a single day.

## **2.5 Statistical analysis**

Analysis of variance was undertaken on each data set to test for concentration differences of cadmium, copper lead and zinc in oyster tissues and sediments between five sites. Turkey's comparison of means was used to test for special variations among the five river mouths.

## **2.6 Collecting of samples**

Samples of oysters and the sediment samples for heavy metal analysis and oysters for condition measurements were collected on the same sampling day.

### **2.6.1 Sampling of oysters for analysis of heavy metals**

Randomly selected individuals from one oyster population were collected hence, it was not possible to collect oysters of the same age or similar genetic constitution. The oysters collected were in size range of 50 – 70 mm in length. A metal chisel and hammer were used to detach oysters from their natural habitats. In order to take the whole content of soft tissues of sampled oysters, measures were taken to prevent damage to the shells, during the sampling.

Efforts were made to ensure that the oysters collected had a similar exposure time to the water in the intertidal zone. Three replicate samples, each with 12 oysters, were collected every month from each site. The majority of sampled oysters were in the 60mm – 70 mm size range at the Mahurangi, Waiake and Wairau river mouths, whereas at the Awaruku and Taiorahi river mouth locations the majority of oysters were in the 50mm to 60 mm size range. Sampled oysters were packed into pre-labelled self-sealing bags and were kept in ice while they were transported to the AUT research laboratory. There the oysters were kept in a freezer, until they were prepared for analysis. The preparation was carried out either the same day or very next day.

#### **2.6.1.1 Preparation of oysters for chemical analysis**

The oysters collected from the sites were washed thoroughly with deionised (DI) water to prevent contamination from the shell surface. Oysters were opened by using a stainless steel oyster knife. The soft tissues of the oysters were removed by using stainless steel scalpel blades. The excised soft tissues of each sample were collected separately into pre-weighed (to the nearest 0.01 mg) porcelain crucibles. The content in each crucible was dried at 60°C for 48 hours (Pridmore, Roper *et al.* 1990) and the crucibles with contents cooled to room temperature in a desiccator. All dried oyster tissue samples were ground manually into fine powder form with a mortar and pestle and each ground sample was stored separately in plastic vials until they were analysed.

#### **2.6.1.2 Chemical analysis of oyster tissues**

There are several methods of sample preparation including acid digestion (Ng 2000). Due to financial restrictions only US-EPA 200.2 method was used to detect the concentration of cadmium, copper, lead and zinc in oyster tissues and in the river sediments. Due to this reason only the certified sediment reference material was analysed as the quality control procedure.



In this study AGAL – 10 (Australian Government Analytical Laboratory) was used as RM. AGAL – 10 RM are sediments of Hawksbury River, in Australia. At every digestion session 0.5gms of reference material was analysed along with the analysis of sediments and oyster samples. AGAL – 10 total acid extractable (TAE) certified values were used as the standard certified values for the concentration of cadmium, copper, lead and zinc. The ICP- AES reading on the AGAL – 10 using US-EPA200.2 method was then checked against the TAE certified values.

Three replicates of 0.5 grams of ground oyster tissues were digested with a 10 ml mixture of nitric acid (1v: 1v) 15: hydrochloric acid (US-EPA 200.2 total recoverable method). The digestion reagent and sample mixture was heated to 85°C for 30 minuets using a digester unit (Velp Scintifica DK 20). The mixture was then diluted to 50 ml with deionised water and analysed by using VARIAN Liberty AX Sequential Inductively Coupled Plasma - Atomic Emission Spectrometer. Table 2.1 shows the concentration values of AGAL – 10 obtained in this study and the TAE certified values ( Louie 1994).

**Table 2.1** Concentration values of AGAL – 10 obtained in this study and the TAE certified values (Louie 1994).

Element	Cadmium( $\mu\text{g/g}$ )	Copper( $\mu\text{g/g}$ )	Lead( $\mu\text{g/g}$ )	Zinc( $\mu\text{g/g}$ )
Total Acid Extractable values	$9.11 \pm 0.65$	$23.51 \pm 1.63$	$42.34 \pm 5.24$	$57.12 \pm 3.08$
Average values obtained by USEPA 200.2 method	$9.09 \pm 0.37$	$24.52 \pm 1.78$	$40.11 \pm 1.14$	$54.13 \pm 6.72$

Statistical evaluation conducted using the Independent t-tests suggests that the mean values for cadmium, copper, lead and zinc are comparable with those of AGAL-10 TAE certified values since the *p*-values for each independent *t* test

is greater than 0.05, there is no significant difference between the standard and the experimental means (Cd **P-Value** = **0.951** , Cu **P-Value** = **0.290** , Pb **P-Value** = **0.399** Zn **P-Value** = **0.229**. Detailed statistical calculations are attached in Appendix 1

## **2.6.2 Sampling of oysters for condition measurements**

Three replicate samples, each with three oysters ranging from 50-70mm in length were collected from the same population for the measure of condition and to calculate condition index. The length of the oyster shell is important in age determination only for the first six months of the life (Rebelo, Amaral *et al.* 2003). As the oysters compete for space with other intertidal organisms, the sizes may vary dramatically with age (Burden 2002). Therefore, the size did not provide reliable information about the age of mature oysters. However, when there is no competition for space, size can indicate the age of the oysters. For this reason attempts were made to collect oysters of a similar length (50-60 mm) from each river mouth in order to minimize the variations in CI due to size class differences. All the collected oysters for this purpose were kept cool to minimise the weight lost due to respiration, and were taken to the AUT research lab for further analyses.

### **2.6.2.1 Preparation of oysters for condition indices measurements**

The attached seaweeds and encrusted organisms on the oyster shells were removed and cleaned before taking the measurements for calculation of CI. The oysters were wiped and the whole animal weights including shell were recorded separately for each oyster. The soft tissues of each oyster were removed and kept separately into pre-weighed (to the nearest 0.01 mg) porcelain crucibles. These crucibles with tissues were dried at 60°C for 48 hours (Pridmore, Roper *et al.* 1990). The oyster shells were dried overnight in the oven at 45°C before they were weighed. After drying, the crucibles with the dried tissues were weighed separately to the nearest 0.01 mg. The dried

tissue weight of each oyster was calculated by subtracting the crucible weight from the crucible with tissue weight

### **2.6.3 Sediment sampling**

As mentioned before, the study aimed to measure short term variations of heavy metal concentrations in bottom sediments at the five river mouths. To achieve this objective, sediments were collected from the topographic surface of the river mouths, as current contaminants deposit mainly on surface sediments (Burden 2002). In order to keep consistency of sampling, the sediment samples were taken within approximated 1 m<sup>2</sup> of fixed points at each river mouth throughout the sampling period.

The three replicate surface sediment samples were collected from the top 1 cm of the surface depth from the river bottoms, at the river mouths of Mahurangi, Awaruku, Waiake, Taiorahi and Wairau. Approximately 250 gm of sediments were collected from each of the river mouths, and placed in previously labelled self sealing bags and brought to the laboratory where they were kept under refrigeration until they were sieved and separated. Water in sediment samples were decanted properly before they were packed for transport to the AUT laboratory to prevent desorption of adsorbed heavy metal compounds of the sediments (Klein 1994).

#### **2.6.3.1 Preparation of Sediments for analysis**

Each sediment sample was initially wet sieved by using 212 µm mesh using DI water. This was done in the very next day of the sampling. Any unwanted material such as shell fragments, coastal organisms and large rock particles were removed from the sediment samples prior to and during sieving. Swirling of sediments with DI water was done within a sieve to facilitate the mechanical separation of smaller particles from larger particles during the sieving. The sieved sediments (particle size >212µm) were dried completely in an oven at a

temperature of 80<sup>0</sup> C (Burden 2002). The dried sediment samples were gently disaggregated with a ceramic pestle, and mixed thoroughly in order to get a composite sample. The ground sediment samples were again sieved by using mesh of 75 µm and were stored in plastic vials until they were analysed.

#### **2.6.3.2 Chemical analysis of sediments**

One gram of ground sediments was digested according to US-EPA method 200.2 for total recoverable methods as describe for the digestion of oyster tissues. In order to keep the analytical expenses within the approved limit of the research budget only one composite sample of the sediment/month/site was analysed.

#### **2.7 Reagents used for the chemical analysis**

A mixture of nitric acid and hydrochloric acid were used for the digestion of ground oyster samples and sieved sediment samples. Standard solutions of 1000ppm with Merck certificate of AA spectroscopy standards were used as stock standard of cadmium, copper, lead and zinc. All reagents used were of analytical grade from Merck, Darmstadt, Germany. The solutions of 1000 ppm were used to prepare standard dilutions of known concentration of 1ppm, 2ppm, 4ppm, 10 ppm 25 ppm, and 50ppm and 100ppm solutions.

#### **2.8 Summary**

Oysters and sediments were sampled from selected localities of the river mouths of Mahurangi, Awaruku, Waiake, Taiorahi and Wairau. Samples were taken each month between November 2002 and October 2003. Oysters and sediments were chemically analysed using ICP-AE and the condition of the oysters were measured using dry flesh weight and shell weight parameters.

## **Chapter 3**

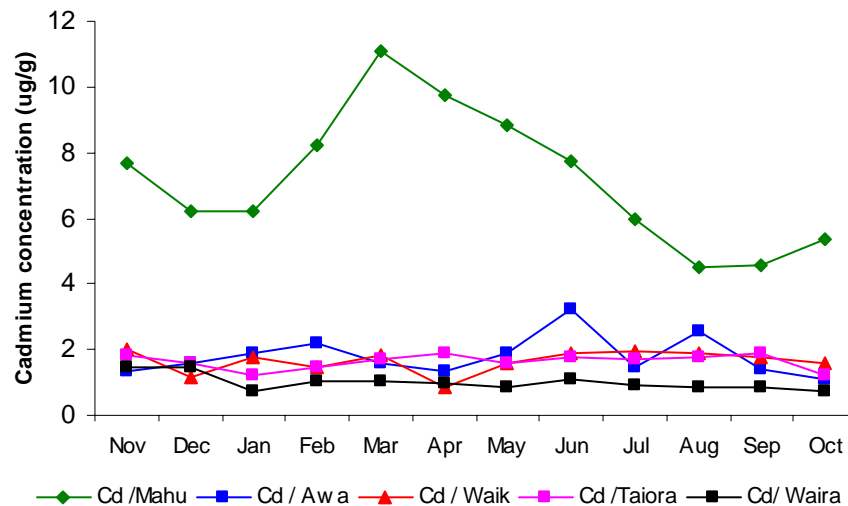
### **3. Results**

This chapter provides the combined results of the chemical analysis of oyster tissues, sediments and the condition of oysters sampled at the studied five river mouths during the period of November 2002 to December 2003.

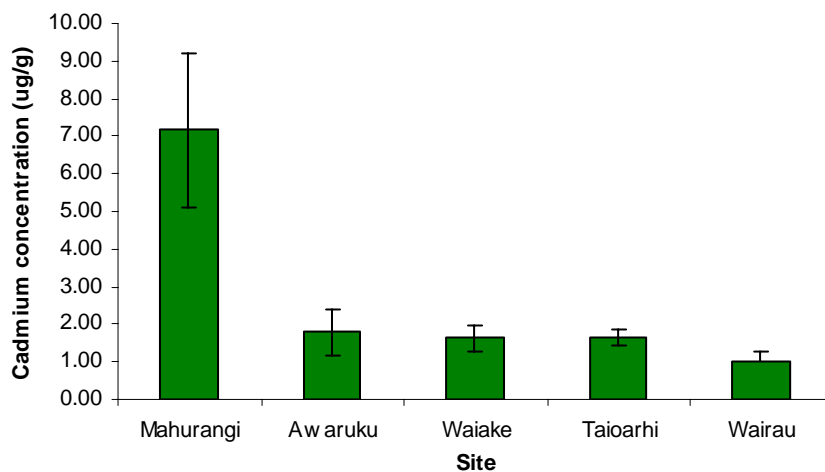
#### **3.1. Results of the chemical analysis of the oyster tissues**

Various concentration patterns of cadmium, copper, lead and zinc were observed in oyster tissues sampled between November 2002 and October 2003 at the five studied river mouths. The concentration of some heavy metals found in oyster tissues showed significant temporal or spatial pattern of variation. Most data sets were not normally distributed because the accumulation of cadmium, copper, lead and zinc in oysters is controlled by a number of environmental, physical and chemical factors

### 3.1.1 Spatial and temporal variations of cadmium concentrations in oyster tissues



**Fig 3.1** Monthly variation of cadmium concentration in oyster tissues at the five river mouths studied during November 2002 to October 2003.



**Fig.3.2** Mean  $\pm$  SD of annual cadmium concentration in oyster tissues sampled at the five river mouths during the period of November 2002 to October 2003.

Mean annual cadmium concentrations in oyster tissues at Mahurangi were higher than the cadmium concentration in oysters of Awaruku, Waiake, Taioarhi and Wairau. These higher values were observed through out the

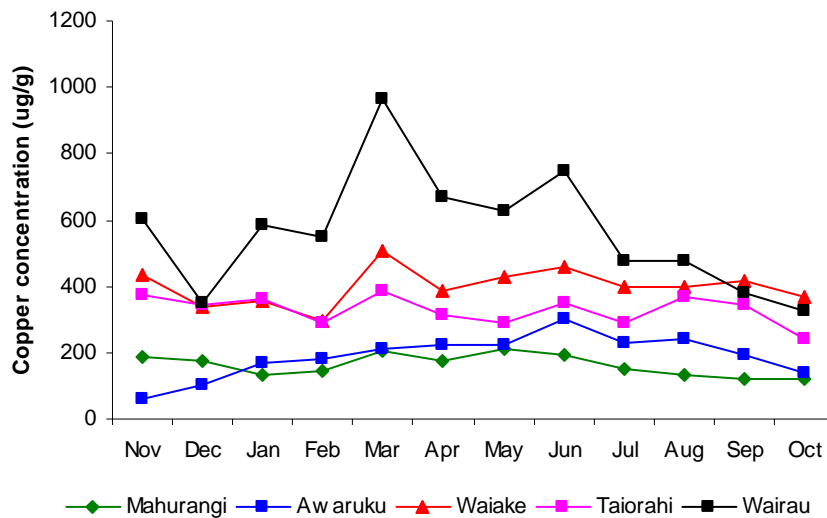


**Table 3.2 Tukey's comparison of means for cadmium**

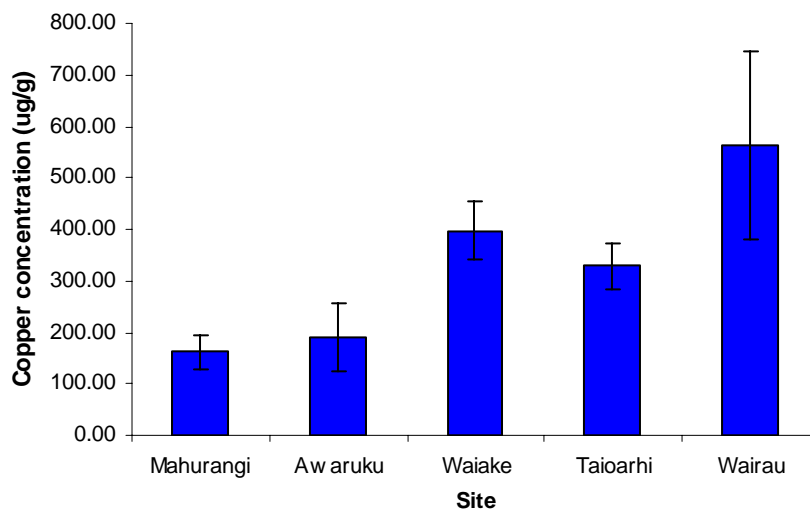
Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	4.8575	0.3018	16.095	<b>Y</b>	0.0000
Awaruku vs Taiorahi	- 0.1792	0.3018	- 0.594	<b>N</b>	0.9755
Awaruku vs Waiake	-0.1742	0.3018	- 0.577	<b>N</b>	0.9779
Awaruku vs Wairau	- 0.7967	0.3018	-2.640	<b>N</b>	0.770
Mahurangi vs Taiorahi	-5.037	0.3018	- 16.69	<b>Y</b>	0.0000
Mahurangi vs Waiake	- 5.032	0.3018	- 16.67	<b>Y</b>	0.0000
Mahurangi vs Wairau	-5.654	0.3018	- 18.73	<b>Y</b>	0.0000
Taiorahi vs Waiake	0.0050	0.3018	0.017	<b>N</b>	1.0000
Taiorahi vs Wairau	-0.6175	0.3018	-2.046	<b>N</b>	0.2584
Waiake vs Wairau	-0.6225	0.3018	-2.063	<b>N</b>	0.2510



### 3.1.2 Spatial and temporal variations of copper concentrations in oyster tissue



**Fig 3.3** Monthly variation of copper concentration in oyster tissues at the five river mouths studied during November 2002 to October 2003.



**Fig.3.4** Mean  $\pm$  SD of annual copper concentration in oyster tissues sampled at the five river mouths during the period of November 2002 to October 2003.

The above results of the monthly copper concentration in oyster tissues indicate that the oyster tissues from the river mouth of Wairau has remarkably higher level of copper during the months of November 2002 to July 2003 ( $350 \mu\text{g/g} \pm 60$  to  $470 \mu\text{g/g} \pm 30$ ) (Fig. 3.3). Between July 2003 and October 2003 copper levels has plateaued out and became close to the levels at Waiake and Taioarhi. Copper levels in oysters at the river mouths of Mahurangi, Awaruku, Waiake

and Taiorahi fluctuate only with in  $63 \mu\text{g/g} \pm 17$  and  $460 \mu\text{g/g} \pm 43$  throughout the sampling period of November 2002 to October 2003. However, the annual mean copper levels in oyster tissues shows the following order; Wairau  $5601 \mu\text{g/g} \pm 182$ . > Waiake  $400\mu\text{g/g} \pm 57$  > Taiorahi  $330 \mu\text{g/g} \pm 44$  > Awaruku  $190 \mu\text{g/g} \pm 65$  > Mahurangi  $160\mu\text{g/g} \pm 33$ . A significantly lower concentration of Cu ( $168.30\mu\text{g/g}$  of total sample) was observed in oyster tissues sampled at the river mouth of the Mahurangi compared to other river mouths. During the sampling period (November 2002 to October 2003) the highest concentration of copper was found in oysters living in the Wairau river mouth. The mean  $\pm$  SD of annual copper concentration in the oyster tissues in this site was reported as  $560 \mu\text{g/g} \pm 182$  of total sample. A Tukey's comparison of annual means showed significant difference in mean annual copper concentration in oyster tissues between Awaruku and Taiorahi, Awaruku and Waiake, Awaruku and Wairau, Mahurangi and Taiorahi, Mahurangi and Waiake, Mahurangi and Wairau, Taiorahi and Waiake, Waike and Wairau ( $p < 0.05$ ), (Table 3.3. & Table 3.4).

**Table 3.3 Results of analysis of variance for copper concentration between sites**

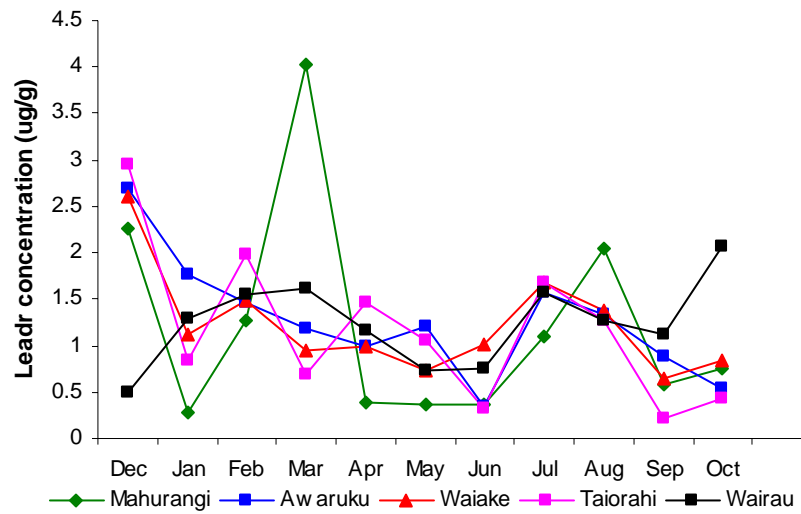
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	4	1249909	1249909	312477	35.67	0.000
Error	55	481771	481771	8759		
Total	59	1731680				

S = 93.5920      R-Sq = 72.18%      R-Sq(adj) = 70.16%

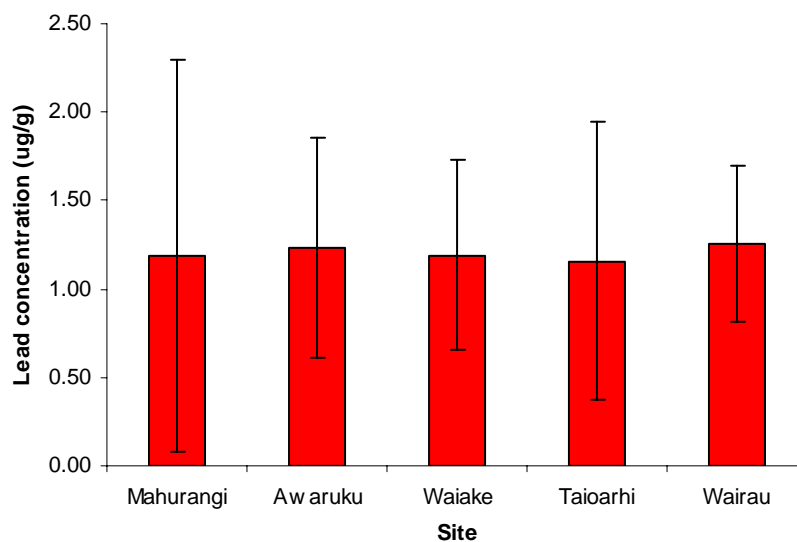
**Table 3.4 Tukey's comparison of means for copper**

Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	-22.96	38.21	-0.6010	<b>N</b>	0.9744
Awaruku vs Taiorahi	135.77	38.21	3.5534	<b>N</b>	0.9768
Awaruku vs Waiake	207.69	38.21	5.4356	<b>Y</b>	0.0000
Awaruku vs Wairau	371.12	38.21	9.7130	<b>Y</b>	0.0000
Mahurangi vs Taiorahi	158.7	38.21	4.154	<b>Y</b>	0.0011
Mahurangi vs Waiake	230.6	38.21	6.037	<b>Y</b>	0.0000
Mahurangi vs Wairau	394.1	38.21	10.314	<b>Y</b>	0.0000
Taiorahi vs Waiake	71.91	38.21	1.882	<b>N</b>	0.3390
Taiorahi vs Wairau	235.35	38.21	6.160	<b>Y</b>	0.0000
Waike vs Wairau	163.4	38.21	4.277	<b>Y</b>	0.0007

### 3.1.3 Spatial and temporal variations of lead concentrations in oyster tissues



**Fig 3.5** Monthly variation of lead concentration in oyster tissues at the five river mouths studied during November 2002 to October 2003.



**Fig.3.6** Mean  $\pm$  SD of annual lead concentration in oyster tissues sampled at the five studied river mouths during the period of November 2002 to October 2003.

The concentrations of lead in the oyster tissues were fluctuated between the ranges of 0.28 µg/g (at Mahurangi in January 2003) and 2.95 µg/g (at Taiorahi in December). March 2003, Mahurangi sample displays the highest abundance of lead for the sampling period. Qualitative field – book observations note the presence of vehicular traffic in the estuary in the days preceding sample collection. No relationship was observed between the monthly variation of the concentration of lead in oyster tissues sampled at the five river mouth habitats during the period of November 2002 and October 2003, (Fig 3.5). Annual mean lead concentrations in oysters at all the five estuaries were very close. The highest and the lowest mean annual concentration values of lead in the oyster tissues were reported at the Mahurangi and Waikere river mouths respectively, and it varied between  $1 \mu\text{g/g} \pm 1.0$  and  $1 \mu\text{g/g} \pm 1.0$ , Fig 3.6. A Tukey's comparison of annual means showed no significant difference in mean annual lead concentrations in oyster tissues between November 2002 and October 2003, ( $p > 0.05$ ), (Table 3.5. & Table 3.6).

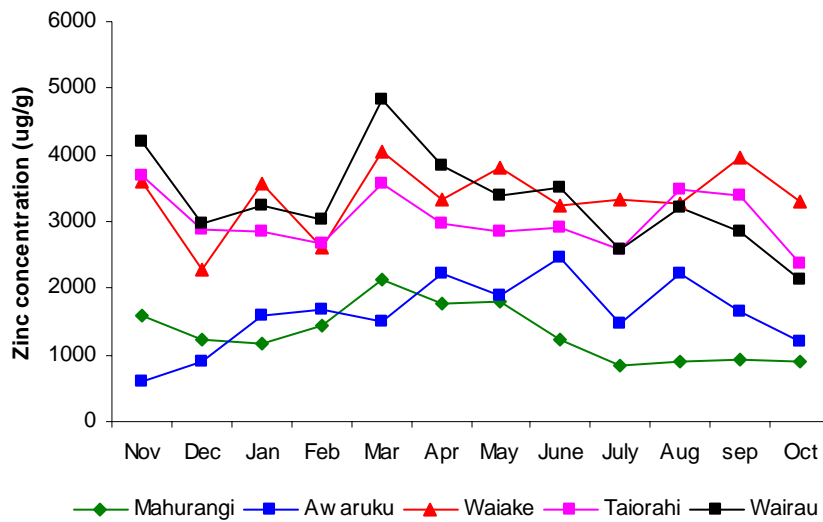
**Table 3.5: Results of analysis of variance for lead concentration between sites**

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	4	0.4682	0.4682	0.1171	0.30	0.879
Error	55	21.7667	21.7667	0.3958		
Total	59	22.2349				
S = 0.629093	R-Sq = 2.11%	R-Sq(adj) = 0.00%				

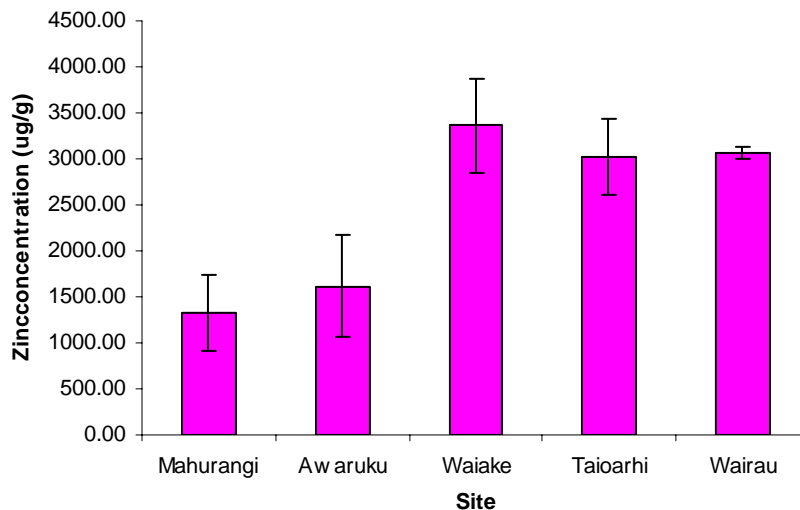
**Table 3.6 Tukey's comparison of means for lead**

Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	-0.2250	0.2568	-0.8761	<b>N</b>	0.9045
Awaruku vs Taiorahi	-0.0742	0.2568	-0.2888	<b>N</b>	0.9984
Awaruku vs Waiake	-0.0408	0.2568	-0.1590	<b>N</b>	0.9999
Awaruku vs Wairau	0.0275	0.2568	0.1071	<b>N</b>	1.0000
Mahurangi vs Taiorahi	0.1508	0.2568	0.5873	<b>N</b>	0.9764
Mahurangi vs Waiake	0.1842	0.2568	0.7171	<b>N</b>	0.9517
Mahurangi vs Wairau	0.2525	0.2568	0.9832	<b>N</b>	0.8617
Taiorahi vs Waiake	0.0333	0.2568	0.1298	<b>N</b>	0.9999
Taiorahi vs Wairau	0.1016	0.2568	0.3959	<b>N</b>	0.9947
Waiake vs Wairau	0.0683	0.2568	0.2661	<b>N</b>	0.9989

### 3.1.4 Spatial and temporal variations of zinc concentrations in oyster tissues



**Fig 3.7** Monthly variation of zinc concentration in oyster tissues at the five river mouths studied during November 2002 to October 2003.



**Fig.3.8** Mean  $\pm$  SD of annual zinc concentration in oyster tissues sampled at the five river mouths during the studied period of November 2002 to October 2003.

Specific pattern was not observed in the concentrations of zinc in oyster tissues at the river mouths of Mahurangi, Awaruku, Waiake, Taiorahi and Wairau during the sampling period. However the mean annual zinc concentrations in oysters at Waiake, Taiorahi and Wairau were very close ( $3021\mu\text{g/g} \pm 420 - 3073 \pm 64\mu\text{g/g}$ ) while the concentrations at Mahurangi and Awaruku varied with in a very narrow range ( $1327 \mu\text{g/g} \pm 419 - 1617 \mu\text{g/g} \pm 550$ ). A Tukey's comparison of annual means showed significant difference in mean annual zinc concentrations between Awaruku and Taiorahi, Awaruku and Waiake, Awaruku and Wairau, Mahurangi and Taiorahi, Mahurangi and Waiake, Mahurangi and Wairau ( $p < 0.05$ ), and the concentrations of zinc in the oyster tissues sampled at the river mouths of Waiake, Taiorahi and Wairau were not significantly different (Table 3.7. & Table 3.8.)

**Table 3.7: Results of analysis of variance for zinc concentration between sites**

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	4	47773347	47773347	11943337	39.44	0.000
Error	55	16654950	16654950	302817		
Total	59	64428297				

S = 550.288      R-Sq = 74.15%      R-Sq(adj) = 72.27%

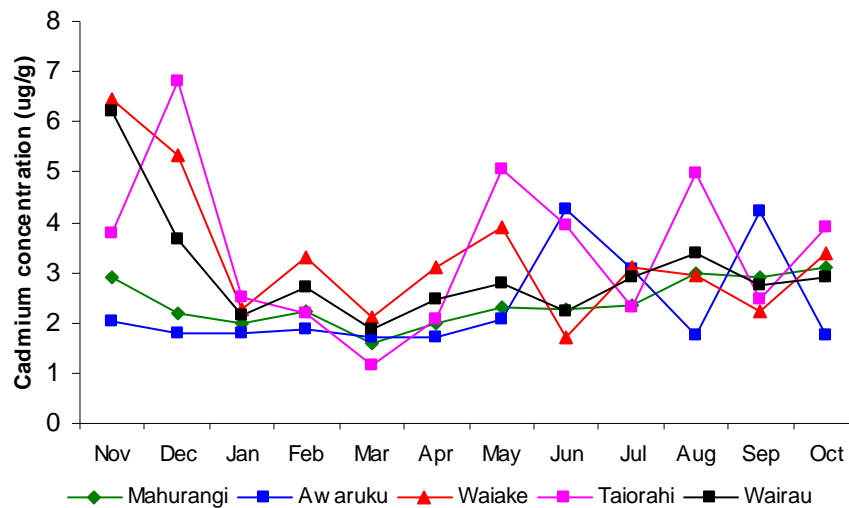


**Table 3.8 Tukey's comparison of means for zinc**

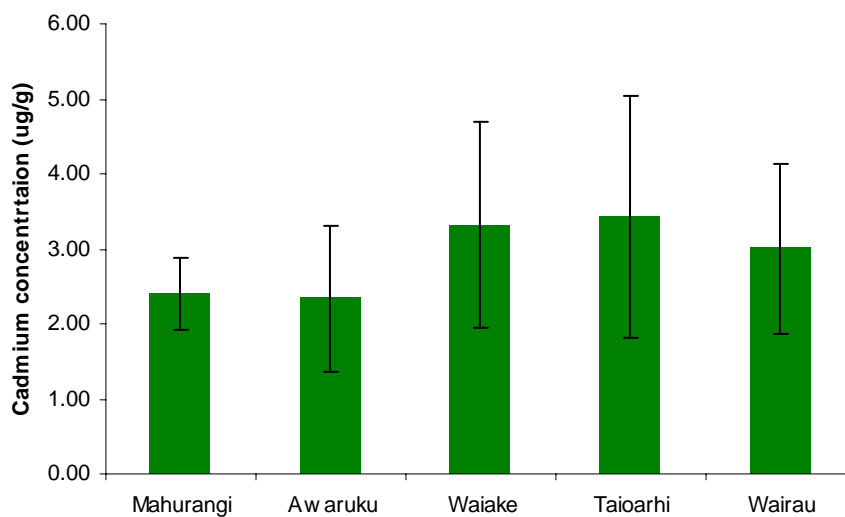
Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	-363.5	224.7	-1.618	<b>N</b>	0.4926
Awaruku vs Taiorahi	1446.2	224.7	6.438	<b>N</b>	0.0000
Awaruku vs Waiake	1661.6	224.7	7.396	<b>N</b>	0.0000
Awaruku vs Wairau	1734.5	224.7	7.721	<b>N</b>	0.0000
Mahurangi vs Taiorahi	1810	224.7	8.056	<b>N</b>	0.0000
Mahurangi vs Waiake	2025	224.7	9.014	<b>N</b>	0.0000
Mahurangi vs Wairau	2098	224.7	9.339	<b>N</b>	0.0000
Taiorahi vs Waiake	215.3	224.7	0.9585	<b>N</b>	0.8723
Taiorahi vs Wairau	288.3	224.7	1.2831	<b>N</b>	0.7026
Waiake vs Wairau	72.92	224.7	0.3246	<b>N</b>	0.9975

## 3.2 Results of the chemical analysis of sediments

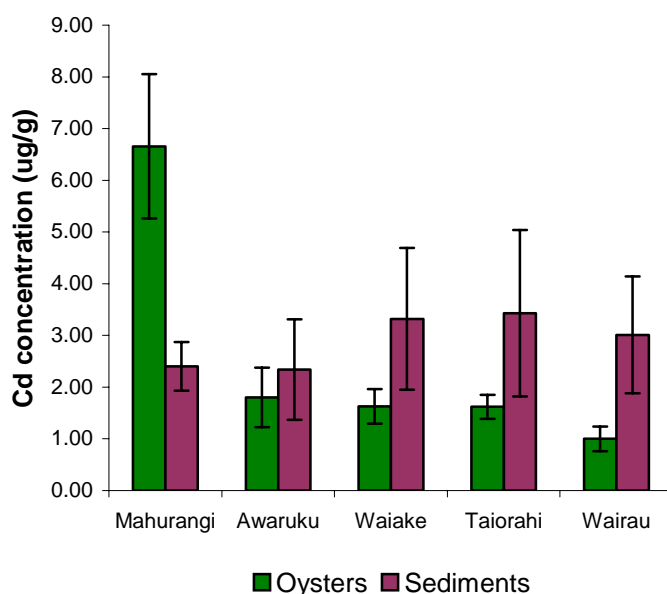
### 3.2.1 Spatial and temporal variation of cadmium concentrations in sediments



**Fig 3.9** Monthly variation of cadmium concentration in sediments at the five river mouths studied during November 2002 to October 2003.



**Fig.3.10** Mean  $\pm$  SE of annual cadmium concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.



**Fig 3.11** Variation of mean annual  $\pm$  SD of cadmium concentration in oyster tissues and sediments sampled at the five river mouths during the period of November 2002 to October 2003.

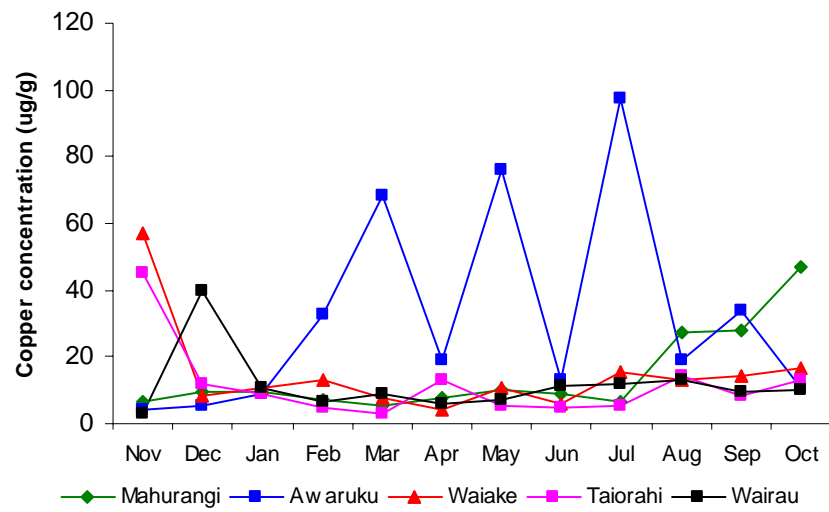
Highest cadmium concentrations in sediments were observed samples collected at the river mouth of Taiorahi. Cadmium concentrations in sediments at Taiorahi reached high values during the months of December 2002, May and August 2003 (Fig 3.9). Lowest value for the concentration of cadmium among the river sediments was also observed in the sediments of Taiorahi in March 2003. Cadmium concentration in sediments at Taiorahi varied within wide concentrations levels. Minimum variations of cadmium levels were observed in the sediments sampled at Mahurangi. A sharp reduction in cadmium concentration was observed in the sediments at Waiake and Wairau between November 2002 and January 2003 (Fig 3.9). Cadmium concentrations were found to be higher in the oyster tissues than in the sediments at the river mouths of Mahurangi and Awaruku, whereas higher concentrations of cadmium were observed in the sediments than the cadmium concentration of the oysters at the river mouths of Awaruku Waiake, Taiorahi and Wairau (Fig. 3.11). Slight correlation between the concentration of cadmium in the oysters and in the sediments was observed ( $R^2 = 0.3235$ ). Annual mean cadmium concentration in sediment sampled at Waiake, Taiorahi and Wairau were higher than the



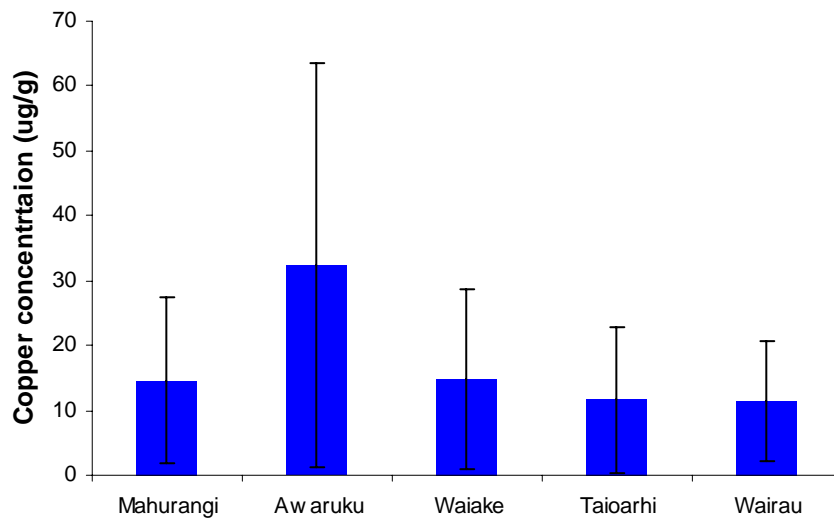
**Table 3.10 Tukey's comparison of means for cadmium in sediments**

Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	0.668	0.4805	0.1391	N	0.9999
Awaruku vs Taiorahi	1.0955	0.4805	2.2801	N	0.1669
Awaruku vs Waiake	0.9813	0.4805	2.0425	N	0.2600
Awaruku vs Wairau	0.6732	0.4805	1.4013	N	0.6295
Mahurangi vs Taiorahi	1.0287	0.4805	2.141	N	0.2179
Mahurangi vs Waiake	0.9145	0.4805	1.903	N	0.3278
Mahurangi vs Wairau	0.6064	0.4805	1.262	N	0.7151
Taiorahi vs Waiake	-0.1142	0.4805	-0.2376	N	0.9993
Taiorahi vs Wairau	-0.4222	0.4805	-0.8788	N	0.9035
Waiake vs Wairau	-0.3081	0.4805	-0.6412	N	0.9676

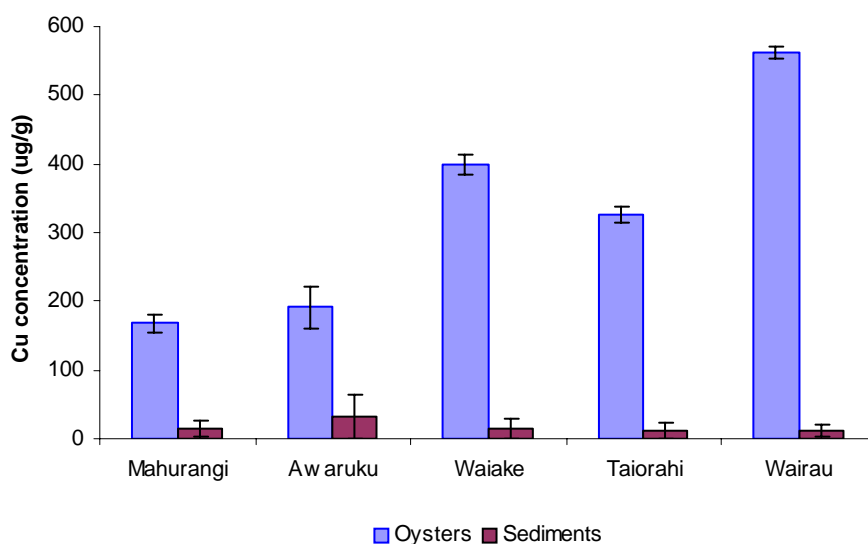
### 3.2.2 Spatial and temporal variations of copper concentrations in sediments



**Fig 3.12** Monthly variation of copper concentration in sediments at the five studied river mouths during November 2002 to October 2003.



**Fig.3.13** Mean  $\pm$  SD of annual copper concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.



**Fig 3.1.4** Variation of mean annual  $\pm$  SD of copper concentration in oyster tissues and sediments sampled at the five river mouths during the period of November 2002 to October 2003.

The results of monthly copper concentrations analysis in sediments at the river mouth of Awaruku between January and October 2003 showed wide fluctuation. Higher monthly concentrations of copper in sediments were observed at Awaruku in March May and July 2003 than the rest of the sampling period, (Fig 3.12). Much lower concentrations of copper were observed in the sediments than the oyster tissues sampled at all the five river mouths (Fig.3.14). The relationship between the concentration of copper in oysters and the sediments was statistically not very strong ( $R^2 = 0.3137$ ). The highest mean annual copper concentration in sediments was observed in sediments sampled at Awaruku ( $32\mu\text{g/g} \pm 31$ ). A Tukey's comparison of annual means showed significant difference in mean annual copper concentrations in sediments between all the river mouths with the exception of between Awaruku and Mahurangi and Taiorahi and Waiake (Table 3.11 & Table 3.12).

**Table 3.11 Results of analysis of variance for copper concentration in sediments between sites**

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	4	1249909	1249909	312477	35.67	0.000
Error	55	481771	481771	8759		
Total	59	1731680				

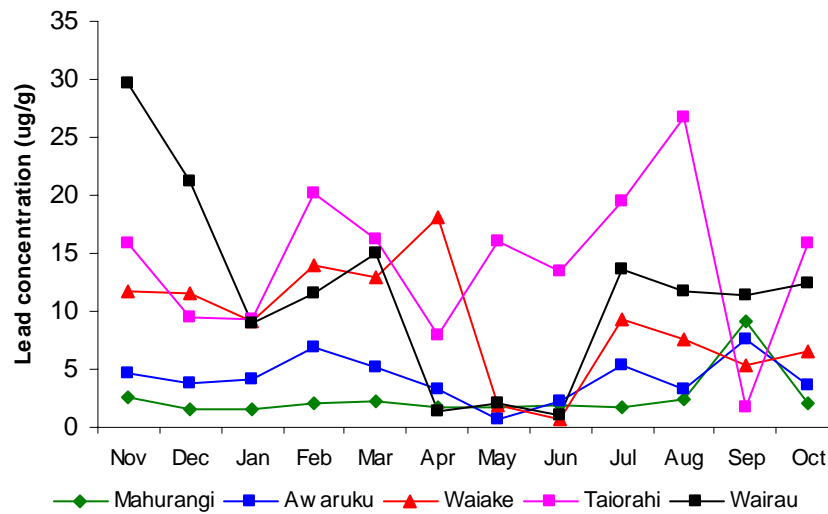
S = 93.5920      R-Sq = 72.18%      R-Sq(adj) = 70.16%



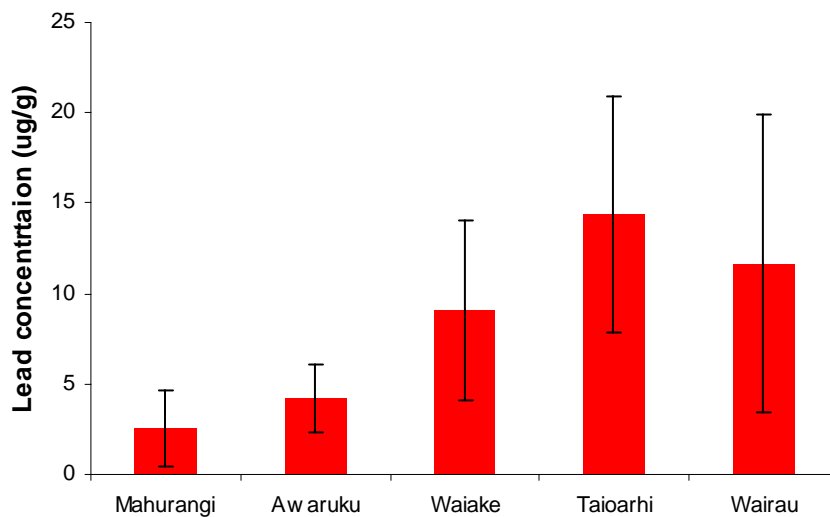
**Table 3.12 Tukey's comparison of means for copper**

Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	-22.96	38.21	-0.6010	<b>N</b>	0.9744
Awaruku vs Taiorahi	135.77	38.21	3.5534	<b>N</b>	0.0068
Awaruku vs Waiake	207.69	38.21	5.4356	<b>Y</b>	0.0000
Awaruku vs Wairau	371.12	38.21	9.7130	<b>Y</b>	0.0000
Mahurangi vs Taiorahi	158.7	38.21	4.154	<b>Y</b>	0.0011
Mahurangi vs Waiake	230.6	38.21	6.037	<b>Y</b>	0.0000
Mahurangi vs Wairau	394.1	38.21	10.314	<b>Y</b>	0.0000
Taiorahi vs Waiake	71.91	38.21	1.882	<b>N</b>	0.3390
Taiorahi vs Wairau	235.35	38.21	6.160	<b>Y</b>	0.0000
Waiake vs Wairau	163.4	38.21	4.277	<b>Y</b>	0.0007

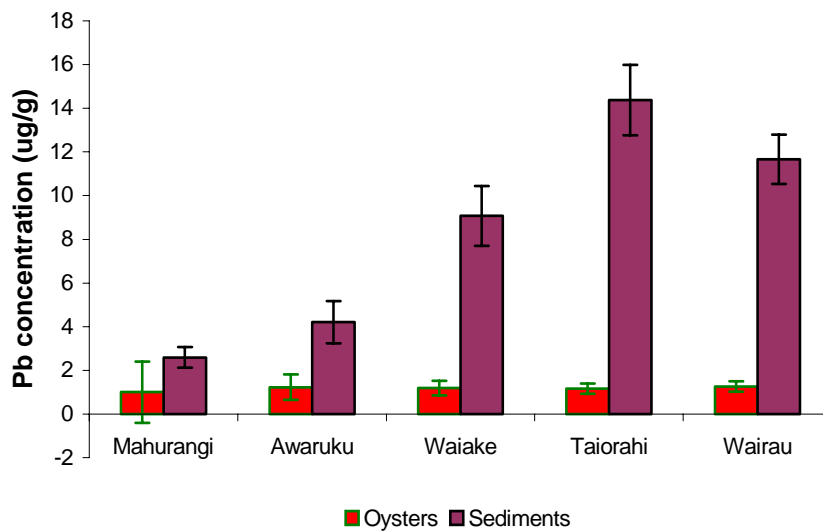
### 3.2.3 Spatial and temporal variations of lead concentrations in sediments



**Fig 3.15** Monthly variation of lead concentration in sediments at the five river mouths studied during November 2002 to October 2003.



**Fig.3.16** Mean  $\pm$  SD of annual lead concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.



**Fig 3.17** Variation of mean annual  $\pm$  SD of lead concentration in oyster tissues and sediments sampled at the five river mouths during the period of November 2002 to October 2003.

No relationship was observed between the monthly variations of the concentration of lead in sediments sampled at the five river mouths (Fig 3.15). Lead concentrations of sediments collected from Taiorahi remains higher than that from the other stations through out the sampling period except in the months of November December 2002 and August 2003. The observed very low concentration in September may be due to an error and may require repetition of the assay. The lead concentrations of sediments at Mahurangi and Awaruku remain consistently lower ( $1.6 \mu\text{g/g}$  and  $9.2 \mu\text{g/g}$ ) than the other stations and the lowest concentrations for lead at these two stations were observed between April to July. Lead concentrations in sediments at Taiorahi and Wairau were vary widely ( $0.7 \mu\text{g/g} - 29.6 \mu\text{g/g}$ ). The highest lead concentration in sediment was observed at Wairau ( $29.6 \mu\text{g/g}$ ) in November 2002. The highest mean annual lead concentration was observed at Taiorahi ( $12\mu\text{g/g} \pm 8$ ) and the lowest ( $3 \mu\text{g/g} \pm 2$ ) was at Mahurangi. Slightly elevated concentrations of lead were observed in the sediments than the oysters at the river mouths of Mahurangi and Awaruku, while the lead concentration in the sediments was much higher than in the oyster tissues sampled at the river mouths of Waiake, Taiorahi and Wairau (Fig 3.17). However, little correlation was found between the

concentration of lead in the oyster tissues and in the sediments ( $R^2 = 0.2401$ ). Tukey's comparison of annual means showed significant difference in mean annual lead concentrations in sediments between Awaruku and Taiorahi, Awaruku and Wairau, Mahurangi and Taiorahi, Mahurangi and Waiake and Mahurangi and Wairau, (Table 3.13 & Table 3.14).

**Table 3.13 Analysis of variance for lead concentration in sediments between sites**

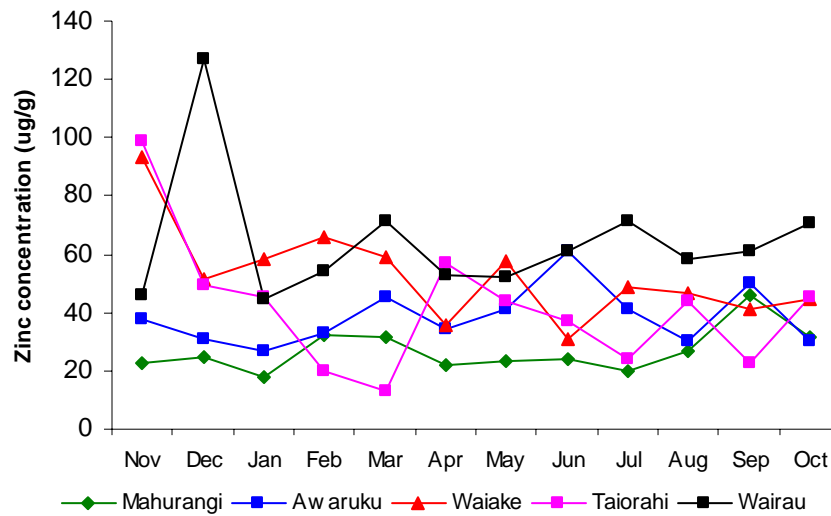
Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	4	0.4682	0.4682	0.1171	0.30	0.879
Error	55	21.7667	21.7667	0.3958		
Total	59	22.2349				

S = 0.629093      R-Sq = 2.11%      R-Sq(adj) = 0.00%

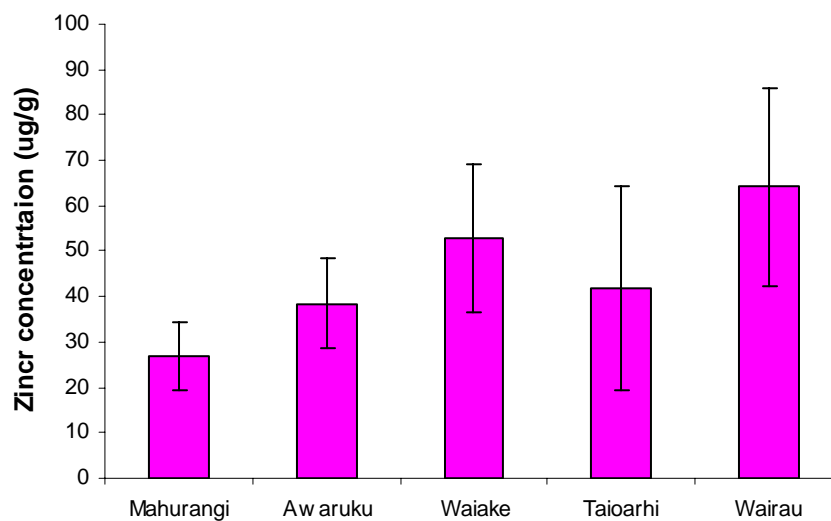
**Table 3.14 Tukey's comparison of means for lead**

Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	-0.2250	0.2568	-0.8761	<b>N</b>	0.9045
Awaruku vs Taiorahi	-0.0742	0.2568	-0.2888	<b>N</b>	0.9984
Awaruku vs Waiake	-0.0408	0.2568	-0.1590	<b>N</b>	0.9999
Awaruku vs Wairau	0.0275	0.2568	0.1071	<b>N</b>	1.0000
Mahurangi vs Taiorahi	0.1508	0.2568	0.5873	<b>N</b>	0.9764
Mahurangi vs Waiake	0.1842	0.2568	0.7171	<b>N</b>	0.9517
Mahurangi vs Wairau	0.2525	0.2568	0.9832	<b>N</b>	0.8617
Taiorahi vs Waiake	0.0333	0.2568	0.1298	<b>N</b>	0.9999
Taiorahi vs Wairau	0.1016	0.2568	0.3959	<b>N</b>	0.9947
Waiake vs Wairau	0.0683	0.2568	0.2661	<b>N</b>	0.9989

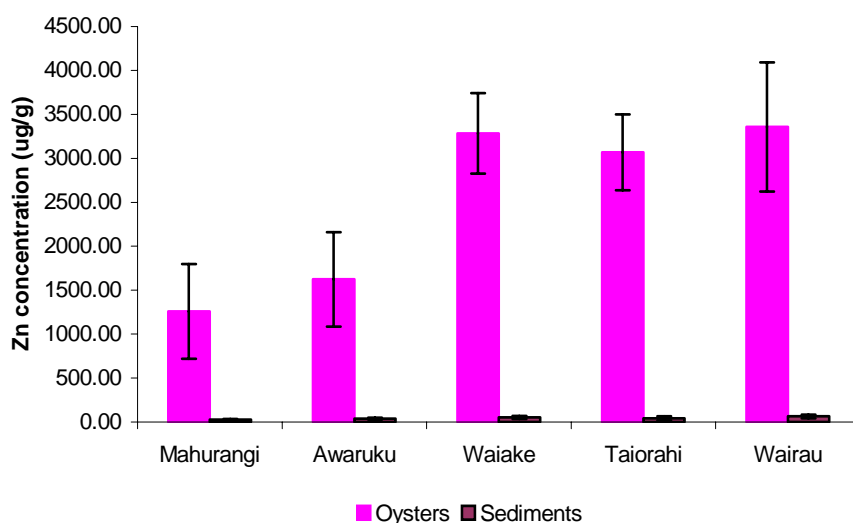
### 3.2.4 Spatial and temporal variations of zinc concentrations in sediments



**Fig 3.18** Monthly variation of zinc concentration in sediments at the five river mouths studied during November 2002 to October 2003.



**Fig.3.19** Mean  $\pm$  SD of annual zinc concentration in sediments sampled at the five river mouths during the period of November 2002 to October 2003.



**Fig 3.20** Variation of mean annual  $\pm$  SD of zinc concentration in oyster tissues and sediments at the five river mouths during the period of November 2002 to October 2003.

No specific pattern was observed in the concentrations of zinc in sediments at the river mouth habitats during the period of November 2002 to October 2003. The monthly variation of zinc concentration in sediments does not show a major difference between the sites. However, the concentrations of zinc in sediments sampled from Waiake and Taiorahi were markedly higher than the concentrations of the other sites. In the month of December zinc concentration in sediments collected from Wairau river mouth were higher than any other month. Zinc concentration levels in sediments collected from all other stations during the period of twelve months remained fairly constant. However the mean annual zinc concentrations in sediments at Waiake, Taiorahi and Wairau were between the range of  $42 \mu\text{g/g} \pm 23$  and  $64 \mu\text{g/g} \pm 22$  while the concentrations at Mahurangi and Awaruku were in the range of  $27 \mu\text{g/g} \pm 8$ – $38 \mu\text{g/g} \pm 10$ . Similar to copper the observed zinc concentrations in oysters were much higher than the copper concentration of the sediments at all of the five river mouths. However, unlike copper, the relationship of concentration of zinc in oysters and in sediments was statistically strong ( $R^2 = 0.7891$ ).

Mean annual zinc sediment concentration at Waiake and Wairau were higher than the zinc concentrations in sediments from other river mouths. Awaruku

and Taiorahi have close sediment zinc concentrations and were higher than that of Mahurangi. A Tukey's comparison of annual means showed significant difference in mean annual zinc concentrations between Awaruku and Wairau, Mahurangi and Waiake, Mahurangi and Wairau ( $p < 0.05$ ) (Table 3.15 & 3.16).

**Table 3.15 Results of analysis of variance for zinc concentration in sediments between sites**

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Site	4	47773347	47773347	11943337	39.44	0.0
Error	55	16654950	16654950	302817		
Total	59	64428297				

S = 550.288      R-Sq = 74.15%      R-Sq(adj) = 72.27%

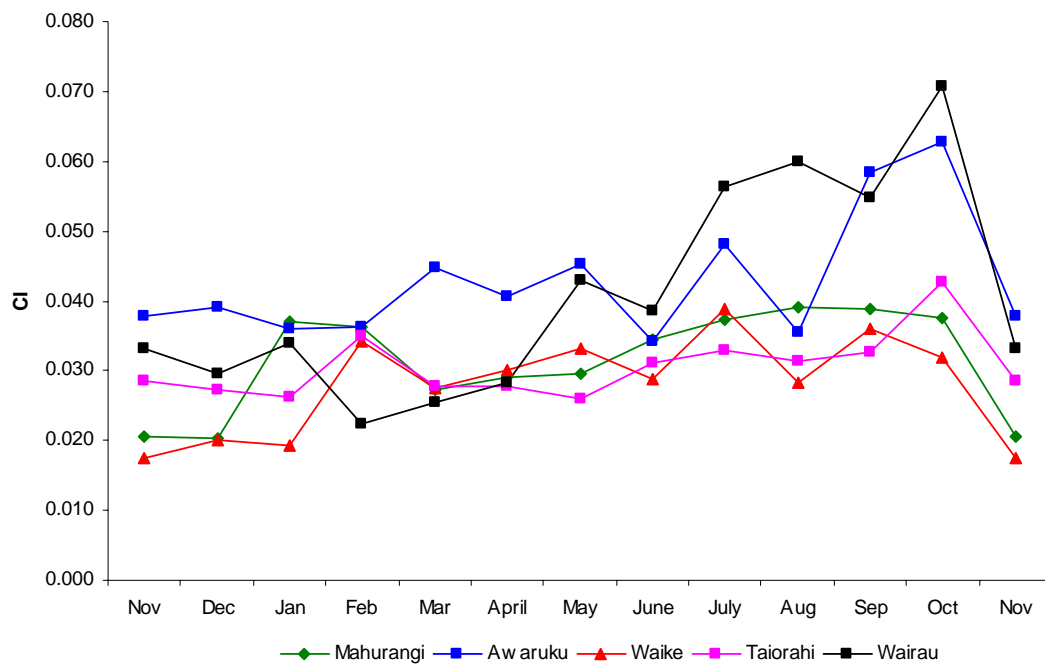


**Table 3.16 Tukey's comparison of means for zinc**

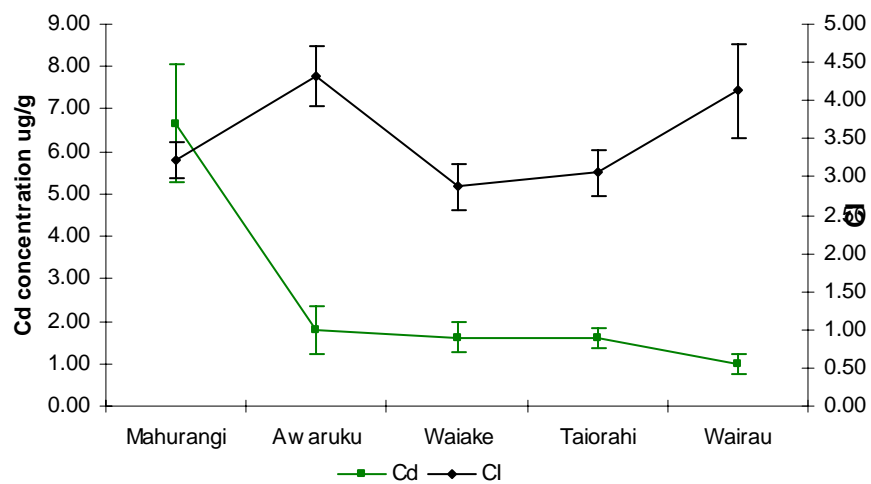
Sites	Difference of Means	SE of Difference	T - value	Significant Y/N	P value
Awaruku vs Mahurangi	-363.5	224.7	-1.618	<b>N</b>	0.4926
Awaruku vs Taiorahi	1446.2	224.7	6.438	<b>Y</b>	0.0000
Awaruku vs Waiake	1661.6	224.7	7.396	<b>Y</b>	0.0000
Awaruku vs Wairau	1734.5	224.7	7.721	<b>Y</b>	0.0000
Mahurangi vs Taiorahi	1810	224.7	8.056	<b>Y</b>	0.0000
Mahurangi vs Waiake	2025	224.7	9.014	<b>Y</b>	0.0000
Mahurangi vs Wairau	2098	224.7	9.339	<b>Y</b>	0.0000
Taiorahi vs Waiake	215.3	224.7	0.9585	<b>N</b>	0.8723
Taiorahi vs Wairau	288.3	224.7	1.2831	<b>N</b>	0.7026
Waiake vs Wairau	72.92	224.7	0.3246	<b>N</b>	0.9975

### 3.3 Results of condition index (CI) measurements

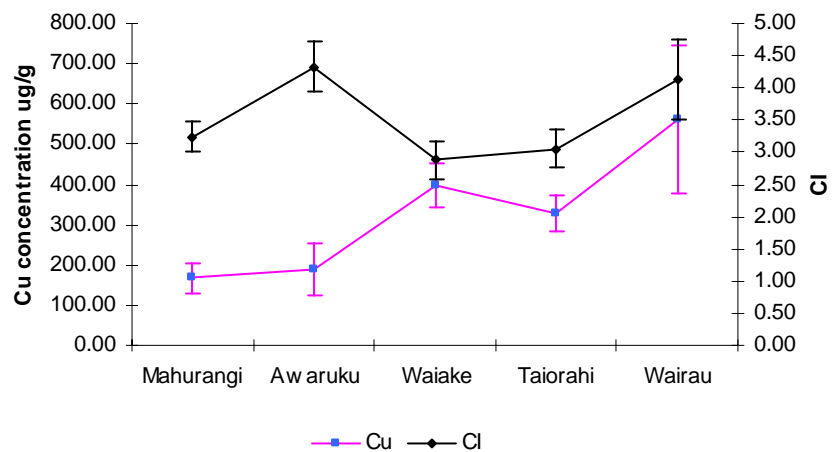
The condition of the oysters shows a wide range of difference spatially and temporally. The condition indices (CI) of the oysters sampled were calculated each month between November 2002 and October 2003.



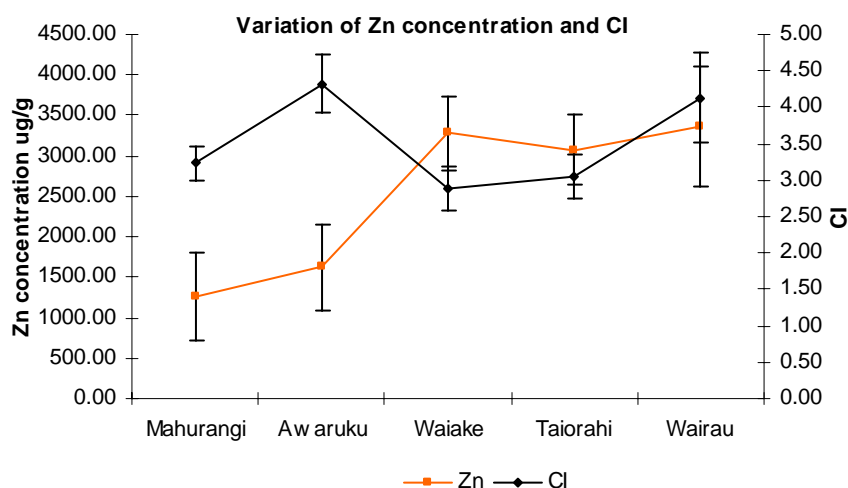
**Fig. 3.21** Monthly variations of CI at the five river mouth habitats between November 2002 and October 2003.



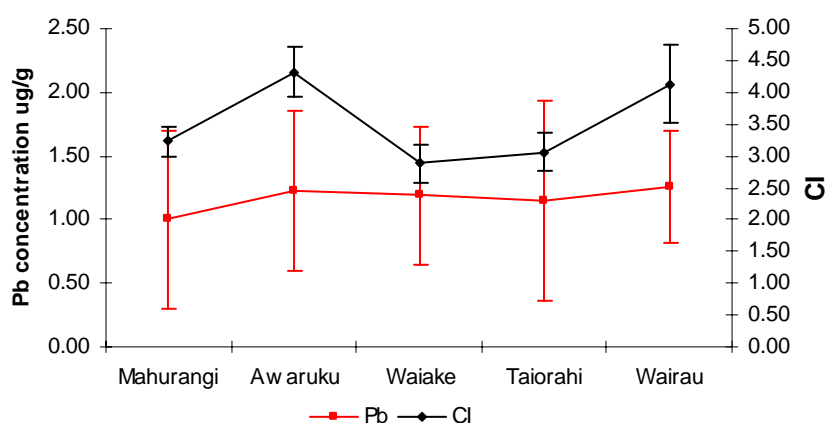
**Fig.3.22** Variation  $\pm$  SD of CI and mean annual cadmium concentration in oysters at five river mouths between November 2002 and October 2003.



**Fig. 3.23** Variation  $\pm$  SD of CI and mean annual copper concentration in oysters at five river mouths between November 2002 and October 2003.



**Fig.3.24** Variation  $\pm$  SD of CI and mean annual lead concentration in oysters at five river mouths between November 2002 and October 2003.



**Fig.3 .25** Variation  $\pm$  SD of CI and mean annual zinc concentration in oysters at five river mouths between November 2002 and October 2003.

Oysters at all the five sites showed lower condition during the period of January to April 2003 than the period of November December of 2002 and May to October 2003 (Fig. 3.17). Oysters at Mahurangi and Awaruku have lowest condition during the months of March and April 2003. Lowest condition of the oysters at Waiake and Taiorahi was seen during January and February, while the

oysters at Wairau showed the lowest condition during the months of February and March 2003 (Fig.3.17). Oysters of all the five river mouth environments showed an increasing trend in condition from May to October 2003. Condition of the oysters at Awaruku was always higher than the condition of oysters sampled at Taiorahi and Waiake. Condition of oysters at Wairau showed a wide range of variation during the sampling period (2.24 in February to 7.07 in October). Condition of oysters at Mahurangi was always lower than the oysters at Awaruku except January and February in 2003. Variation of CI of oysters at

all the five river mouths showed no relationship with the pattern of variation of cadmium concentration in oyster tissues (Fig. 3.18). The r-square value was 0.0785. CI at Awaruku and Wairau was higher than the CI at Mahurangi. But cadmium concentration at Mahurangi was higher than that of Awaruku and Wairau. Even though cadmium concentration at Waiake and Taiorahi were close to Awaruku and Wairau the CI at Wairau was much lower than that of Awaruku and Wairau. CI and copper concentration of oysters at the five river mouths showed no relationship ( $R^2 = 0.0068$ ) (Fig. 3.19). Poor correlation ( $R^2 = 0.3041$ ) was observed between lead concentration and CI at the five sites 3.20. Oysters showed higher CI at the river mouths of Mahurangi and Awaruku where zinc concentrations were lower than the other sites 3.21.

## **Chapter 4**

### **4. Discussion**

As summarised in the above chapter a wide variation of heavy metal content of oyster tissues and sediments, have observed among the studied river mouth habitats. Therefore the main aim of this chapter is to establish relationship between the land use of the river catchments and heavy metal concentrations in selected estuaries.

#### **4.1 Variation of heavy metals in oysters and in sediments**

Concentration of cadmium in oysters at Mahurangi river mouth exceeded the prescribed limits for the human consumption of shellfishes. Lead concentrations in oysters sampled from all the studied estuaries were lower than the permitted concentration for human consumption. Elevated levels of copper and zinc observed in the oyster tissues in the river mouths of Mahurangi, Awaruku, Waiake, Taiorahi and Wairau were found to be in greater concentrations than those of permitted concentrations for consumption by Australian New Zealand food standard code Table 4.1.

Metal	Permitted maximum concentration level	Annual mean concentration level found in this study	
Cadmium	2 µg/g	Mahurangi	7.17 µg/g ± 2.05
		Awaruku	1.79 µg/g ± 0.60
		Waiake	1.64 µg/g ± 0.36
		Taiorahi	1.64 µg/g ± 0.23
		Wairau	1.00 µg/g ± 0.25
Copper	5 µg/g	Mahurangi	162.66 µg/g ± 33.00
		Awaruku	190.86 µg/g ± 64.91
		Waiake	397.65µg/g ± 56.72
		Taiorahi	328.28 µg/g ± 44.21
		Wairau	562.81 µg/g ± 182.02
Lead	2 µg/g	Mahurangi	1.19 µg/g ± 1.10
		Awaruku	1.23 µg/g ± 0.62
		Waiake	1.19 µg/g ± 0.54
		Taiorahi	1.16 µg/g ± 0.79
		Wairau	1.26 µg/g ± 0.44
Zinc	130µg/g	Mahurangi	1327.53 µg/g ± 419.36
		Awaruku	1616.56 µg/g ± 546.69
		Waiake	3365.10 µg/g ± 510.02
		Taiorahi	3020.69 µg/g ± 420.78
		Wairau	3072.61 µg/g ± 64.29

**Table 4.1** Permitted maximum concentration levels of metal contaminants in Molluscs (Australia New Zealand Food Standard Code 2002).

The concentration of cadmium, copper, lead and zinc in oyster tissues and in sediments could provide an indication of the degree of pollution in the studied river mouth environments.

The highest level of cadmium has been observed in the river mouth of Mahurangi, compared to other sites. The major environmental difference observed among the sites was Mahurangi estuary which is surrounded predominantly by farm lands but other estuaries are either urbanised or industrialised. Higher levels of cadmium could be expected from urban and industrialised areas than from unurbanised or non-industrialised areas. However, oyster tissues sampled at Mahurangi where the catchment was predominantly farmlands has the highest level of cadmium. An attempt to rationalize further the higher cadmium concentration in agricultural land, revealed that the phosphoric fertilizers used in pasture lands could be the main cause of this observed high cadmium concentrations in oyster tissues. The concentration of cadmium in the phosphoric fertilizers is often greater than 10 mg/g. Cadmium in phosphoric fertilizers are actively transported to the drainage systems by leaching from acidic soil and erosion (Butler and Timperley 1996). According to Butler and Timperley (1996), pasture soil in Mahurangi is slightly acidic, pH 5.3 – 5.4. Consequently high cadmium levels reaching the Mahurangi river mouth habitats by mobilisation through acidic soil and erosion of surrounding pasture lands could be bioaccumulated within the tissues of oysters during the filtering process. The filtering process of bivalves is very efficient and has the ability to filter water up to 20 liters of water per hour. Butler and Timperley (1996) also have shown the occurrence of high cadmium concentration in oyster tissues at the Mahurangi estuary. Therefore the use of phosphoric fertilizers in pasture lands may contribute to elevated cadmium concentrations in oysters.

Copper levels in oyster tissues at Mahurangi and Awaruku were the lowest out of the five sites monitored values. These estuaries are surrounded by farmlands and residential areas respectively. There are very little or no industries identified in these areas. The lower levels of copper could therefore be related to less commercial land use in the respective areas. The annual mean copper levels at Taiorahi and Waiake were higher than the Mahurangi



and Awaruku but lower than Wairau. Catchments of these two rivers are categorised as residential areas by Roberts (2000). The copper spouting and vehicles could contribute to the copper contaminant in the environment (Hickey 1992).

The observed lead concentrations in oyster tissues at five estuaries were very similar and were not significantly different among five sites. The concentration levels of lead in the oyster tissues were the lowest among all heavy metals in the oysters in this study. The main source of lead of these estuarine environments would be the stormwater collected from catchments of the respective river mouth habitats. This suggests that lead would be the lowest pollutant out of the four metals studied in urban and industrial areas. The most possible reason for these lower levels of lead than cadmium, copper and zinc in the oyster tissues, at urban and industrial catchments would be the gradual removal of lead from New Zealand petrol over the last fifteen years. No differences among sites and low overall concentrations of lead may mean that the major lead source (petrol) has been removed and now there is little contamination from other sources. According to Thompson (1982), environmental lead concentration is lower than the environmental concentration of cadmium, copper and zinc. These low levels of environmental lead concentrations compare to environmental concentrations of cadmium, copper and zinc may be another reason for the observed low lead concentrations in oyster tissues. Slow accumulation rate of lead in the tissues of some species of oysters could also be attributable to the observed low levels of lead (Shulkin Presley *et al* 2003). However sample collected in March 2003, displayed greatest abundance of lead. It was noted that vehicles were driving in the estuary adjacent to the sample site. It was also noted that in the summer periods recreational use of the estuary increased. The ramification of these events may have resulted in increased resuspension of benthic sediments thus allowing adsorbed lead on sediments to become available in the water column (Taylor, Lunn *et al.* 2003).

The low mean annual zinc concentrations have been seen in oysters at the river mouths of Mahurangi and Awaruku. As discussed earlier Mahurangi is solely a farmlands while Awaruku has more dwellings. Even though there is no significant difference between the mean annual zinc concentrations in oysters at the two river mouth habitats, the higher mean annual concentration of zinc in oysters at Awaruku river mouth could be due to the influence of residential activities of the catchment. Galvanized roofing sheets act as major source of zinc in residential areas (Hickey 1992). Therefore the difference seen could be attributed to the difference in number of dwellings at Awaruku catchment. Higher annual mean zinc levels were observed in the habitats of the river mouths of Waiake, Taiorahi and Wairau. Catchment of these river mouths are more urbanised and industrialised than the catchment of Mahurangi and Awaruku. Even though there is no significant difference among river mouths of Waiake, Taiorahi and Wairau, Taiorahi has the lowest annual mean zinc level. These results therefore clearly show the relationship between pollution and the land use of the catchments of these estuaries. These data strongly support the view that zinc levels in oyster tissues can be used as a reliable index of zinc pollution in the estuaries. Results of the this study is also supported by the idea of Shulkin, Presley (2003), who suggests that higher concentration of zinc in oysters living coastal areas close to heavily populated cities is a common problem of pollution in the coastal areas of the world.

High concentrations of copper and zinc in oyster tissues have been observed in this study and are supported by the results of number of studies conducted by locally and internationally. Roper *et al* (1991), found higher concentration of Cu and Zn in oyster tissues sampled in highly polluted Granny's bay site of the Manukau harbour (1219  $\mu\text{g/g}$  and 4146  $\mu\text{g/g}$  respectively), than the copper and zinc level in the oysters sampled at the less polluted Cornwallis site (178 $\mu\text{g/g}$  and 970 $\mu\text{g/g}$  respectively). Timperely (2001), observed higher concentration of zinc than copper in stormwater in urban areas of Auckland (Timperly 2001). This higher concentration of zinc than the copper in stormwater could account for the observed higher concentration of zinc than copper in oyster tissues sampled at the studied river mouth habitats. Because

according to Timperely (2001), stormwater is one major source of copper and zinc contaminants in urban river waters. A study conducted by Hays *et al.* (1998), in Botany Bay, Australia showed a similar concentration pattern of copper and zinc in oysters, and they found much higher concentration levels of copper and zinc in more polluted localities than the reference site of their study in the Botany Bay.

The sediments act as the principal reservoir and carrier of heavy metals in coastal areas. The measured heavy metal content in the sediments was used to evaluate the degree of cadmium, copper, lead, and zinc pollution in these river mouths. Because the heavy metal contaminants take a long period of time to accumulate in the sediments (Phillips and Rainbow 1992), it was difficult to detect temporal variations within the twelve months period of this study. Therefore, only the spatial variation of the concentration of cadmium, copper, lead and zinc in the sediments of the river mouths of Mahurangi, Awaruku, Waiake, Taiorahi and Wairau were examined. The measured heavy metal concentrations of these river sediments would help detect the degree of pollution of adjacent lands of these river mouths. Because sediments show a high capacity to accumulate and integrate heavy metals in the river water (Forstner and Wittmann 1979),

Higher concentrations of cadmium, copper, lead and zinc were observed in the sediments of Waiake, Taiorahi and Wairau compared to the sediments of Awaruku and Mahurangi rivers. The higher concentrations of these metals in Waiake, Taiorahi and Wairau sediments reflect the highly urbanised and industrialised land use of the catchment area of these rivers compared to less urbanised catchments of Mahurangi and Awaruku rivers.

The observed concentration of lead in the sediments of Waiake, Taioarhi and Wairau rivers may originate from urban and industrial wastewaters of the respective catchment areas (Soares, Boaventura *et al.* 1999).

Observed lower mean annual concentrations of lead was lower than the annual mean concentrations of cadmium, copper and zinc in the sediments. It can be

postulated that the gradual removal of the sources of the contaminants, for example removal of lead from petrol used in New Zealand over the past decades may have a positive impact on the pollutant level. Reduced use of a compound with a heavy metal leads to lower the concentration of that heavy metal in sediments (Forstner and Wittmann 1979). However, the results reported by Kuschel (2001), shows unexpectedly high levels of particulate lead in roadside sediments. But this report does not confirm the source of lead even though these suggest that the non-vehicular sources could be the major contributor. van Roon (2000) in a different study noticed similar lead levels in Big Muddy Creek and Mangere Inlet of Manukau Harbour. The catchment of Big Muddy Creek is predominantly bush covered while in Mangere inlet is highly contaminated region. According to van Roon (2000) lead concentration in Mangere Inlet was  $39.42 \mu\text{g/g} \pm 5.134$  and that of Big Muddy Creek was  $10.59 \mu\text{g/g} \pm 2.51$ , which has high traffic density. Therefore it is essential to distinguish lead in petrol from lithological lead before making a conclusion about the origin of this lead.

The concentration of lead and zinc in the sediments of Mahurangi and Awaruku rivers were lower than the lead and zinc in the sediments of the other three river mouths. The lower amount of lead and zinc in the sediments of the Mahurangi and Awaruku rivers could be a result of the less urbanised land use of the catchment of these two rivers.

Cadmium concentration in the sediments collected from the Mahurangi river mouth showed more elevated level than that of Awaruku. Therefore it could be confirmed that the heavy use of fertilizers with high cadmium content in the catchment pasturelands of the Mahurangi river is the reason for higher levels of cadmium seen in sediments collected from Mahurangi estuary (Butler and Timperley 1996).

The concentrations of cadmium, copper, lead and zinc in the sediments of the studied five river mouths were lower compared to these heavy metal concentration values of some coastal areas given in the literature. The concentration of cadmium, copper, lead and zinc obtained in studies conducted

in the coastal areas of Manila bay Philippines, (Prudente, Ichihashi *et al.* 1994). Darwin Harbour ,(Peerzada and Rohoza 1989) Australia and Pasajes Harbour Spain (Legorburu and Canton 1991) were higher than the concentration found in the river mouths of this study. However the order of magnitude of the concentration of cadmium, copper, lead and zinc of those studies found to be similar to this study. The observed difference of the heavy metal concentration in these river sediments appear to be related to the level of human activity on the catchments and the different rates of mobilisation and the movement of the various trace elements.

The measurements of heavy metal concentration in sediments and oyster tissues in this study are used to investigate the relationship between the metal concentration in sediments and oyster tissues.

Cadmium levels in oyster tissues and sediments collected from Mahurangi were different to other four sites. The oyster beds were situated close to marine side of the estuary in which the sediments are always exposed to seawater. This is a major physical difference between Mahurangi estuary and other studied river mouths. According to Butler and Timperley (1996), cadmium adsorbed to fresh water sediments becomes soluble when the sediment is exposed to seawater. Butler and Timperley (1996), whose study carried out at Mahurangi Harbour, showed a substantial decrease in the concentration in particulate bound cadmium as proportion of seawater increased. Therefore when the sediment is less exposed to seawater, the desorption of cadmium would be minimal and as a result higher level of sediment cadmium could be expected. It could therefore be argued that cadmium levels in sediments collected from the river mouths of Awaruku, Waiake Taiorahi and Wairau could be due to less exposure of sediments to seawater. The study carried out by Huanxin, Lejun *et al.* (2000), has showed that cadmium in oyster shells is higher than that in tissues compared to sediments. Because cadmium and calcium have similar geochemical properties, cadmium could replace calcium from oyster shells(Huanxin, Lejun *et al.* 2000). Huanxin, Lejun *et al.* (2000), have suggested that cadmium in shell materials could be derived from bioaccumulated cadmium in soft tissues of oysters. Even though the present study does not determine the heavy metals in

oyster shells it could be argued that less cadmium seen in soft tissues may be resulting from shell calcium been replaced by bioaccumulated cadmium in soft tissues. Lower cadmium levels seen in oyster tissues collected from the river mouths of Awaruku, Waiake, Taiorahi and Wairau may be due to the tissue cadmium being used for deposition in shells. As discussed earlier the cadmium levels in Mahurangi estuary originates from the fertilizers used in surrounding pasture lands. The cadmium levels seen in Mahurangi pasture soil, the freshwater sediments and the sediments from the riverine part of the estuary were 0.3 – 0.4 mg/kg and decreased to 0.02 mg/kg at the Huawai Bay near the mouth of the Harbour (Butler and Timperley 1996). This observation support the view that higher levels of cadmium originating from phosphotic fertilizers used in Mahurangi pasture lands increase the soluble form of cadmium in the surrounding seawater of oyster beds which then facilitate the increased accumulation of cadmium in the soft tissues of oysters. According to Butler and Timperley (1996), the more the sediment is exposed to seawater the more cadmium desorption occurs. Therefore lower levels of cadmium seen in sediment collected from Mahurangi compared to other river mouth habitat could be due to desorption of cadmium from sediments as a result of its longer exposure to seawater. The desorbed cadmium will become part of the bioavailable cadmium pool in the surrounding waters, which could then be taken up by filter feeders. This also could be a reason for observed high concentration of cadmium in oysters in the river mouth of Mahurangi. As discussed above a part of this cadmium may be deposited in oyster shells. The cadmium levels in oyster shells were not determined in this study, and therefore the fate of deposited cadmium in oyster soft tissues cannot be confirmed. However, further studies are necessary to make a conclusion about the tissue cadmium being deposited in oyster shells. Copper concentrations of oyster tissues are found to be higher than that of sediments. A similar relationship was seen in zinc concentrations in oyster tissues and sediments. Similar relationship of copper and zinc concentrations between oyster tissues and sediments has been observed by Huanxin *et al.*, (2000). Oysters are capable of accumulating copper and zinc in 25 – 87 times respectively in their tissues than that are in sediments (Huanxin, Lejun *et al.* 2000). The ability of oysters to accumulate higher concentrations of copper and zinc without affecting biological functions

could be an advantage to the animal in this situation (Viarengo 1985). Occurrence of copper and zinc present in higher concentrations in oysters than in the sediments of these five rivers may be explained as the presence of higher heavy metal content in soluble form in water than in the sediments. This argument can be supported by Scanes (1993), who observed that the immediate source of metals for bivalves is the water soluble metals. However, it is not possible to confirm this hypothesis because, at present, detailed inventories of discharges of cadmium, lead, copper and zinc to these rivers do not exist and no detailed studies of elemental level in water around these river mouths have been conducted. The different concentration levels of cadmium, copper, lead and zinc in oysters and sediments indicate that the concentration of heavy metals in oyster tissues represent the bioavailable fraction of the heavy metals while the concentration in surface sediments provide only an estimation of recent heavy metal levels of the river mouth environments. The results of this study showed that the maximum concentration of copper, lead and zinc were reported at the river mouths of Wairau which received run-offs from heavily urbanised catchment. Similar results were observed by Phillips and Yim (1981), in a heavy metal pollution study conducted in a coastal area of Hong Kong. The observed lead concentrations in oyster tissues were lower than that in sediments in all five sites studied. Therefore the mechanism of lead accumulation in oyster tissues may be contrasting to the mechanism of copper and zinc accumulation. Similar observations have been reported by Huanxin, Lejun *et al.* (2000), and it could therefore be argued that has resulted in lower levels of lead in oyster tissues compared to that in sediments is due to slower accumulation rate of lead by oyster tissues.

Certain studies have detected relationships between the metal concentration in sediments and bivalves for various heavy metals (Phillips and Yim 1981). Forstner and Wittmann (1979) observed linear relationship between cadmium and copper concentration in *Crassostrea gigas* and the concentration of the same element in sediments, in a study conducted in Tasmania. However, Huanxin *et al.* (2000), found no simple linear relationship between metal concentration in sediments and bivalves.

Results from this study show no relationship between heavy metal concentrations in the oyster tissues and the adjacent sediments except for zinc. Therefore it can be suggested that the metal concentrations in the sediment at the study sites do not directly influence the levels of bioavailable metals in aquatic environment except for zinc.

A major limitation of this study was the lack of control over various environmental conditions. An appropriate control would have to have been conducted in the laboratory where oysters and known quantities of heavy metal concentrations could have been added. However, the logistical and time limitations of this study, made these experiments impossible to conduct. Furthermore, the sampling of natural populations with their high level of bio variability caused difficulties in interpretation of data. These limitations could be overcome in future research by increasing the sample sizes and frequency of sampling.

#### **4.2. Variation of Condition Index of oysters**

The condition of the bivalves usually reflects the condition of the environment. Unfavourable environmental factors often cause a physical stress on the animals which is observed through reduced condition indices (Roper, Pridmore *et al.* 1991). The CI calculated in this study were vary temporally and spatially Fig 3.21. The calculated CI at each site was compared with the results obtained from the chemical analysis of the oyster tissues at their respective site to determine the impact of heavy metal concentrations and the health of the oysters.

As discussed earlier the highest level of cadmium was observed from oyster tissues at Mahurangi does not show significant difference on CI compared to CI of the oysters at the other studied river mouths as well there was very poor correlation ( $R^2 = 0.0785$ ) between cadmium concentration in oyster tissues and CI at all five sites. Similar to cadmium there was no strong correlation exist between lead copper and zinc concentrations in oyster tissues and the condition



of oysters (  $R^2$  values lead, copper and CI are 0.3041, 0.0068, 0.0502 respectively).

To investigate the effect of cadmium on the condition of the oysters, the annual mean CI and the annual mean cadmium levels at respective stations were compared. Mahurangi has the highest cadmium level among all sites and CI was lower than Awaruku and Wairau. At Awaruku, Taiorahi and Wairau there was an inverse relationship between CI and cadmium levels, as expected. However, lowest CI was found at Waiake than four other river mouths habitats. Even though statistically no correlation between CI and cadmium levels it could be argued that cadmium may have played a role in condition of oysters.

The annual mean lead concentration in oyster tissues and the annual mean condition values were compared as an attempt to find if lead concentration has any influence on the condition of oysters. No relationship between lead and CI was found at all the examined five river mouths. It could be therefore concluded that lead levels in oysters could not be a factor affecting the condition of oysters, at least at the lead concentration measured.

Throughout the sampling period copper and zinc concentration levels in the oyster tissues sampled at Waiake, Taiorahi and Wairau were higher than that in oysters at Mahurangi and Awaruku. Copper and zinc concentrations in oysters at Mahurangi and Awaruku fluctuated closely within a narrow range (3.3  $\mu\text{g/g}$  and 3.7  $\mu\text{g/g}$ ). Copper and zinc concentrations in oysters sampled at Waiake, Taiorahi and Wairau vary in a narrow concentration range but less than that of Mahurangi and Awaruku. If the condition of the oysters were affected by these metal ions it would have expected closely related CI values in Mahurangi and Awaruku and CI in Waiake, Taiorahi and Wairau should lie within a narrow range. But the observed results do not reflect such a pattern. Therefore it could be concluded that condition of the oysters is not affected by zinc and copper in the environment. Even though changes in condition may not have been brought about by these heavy metals as a single pollutant, it can be therefore be inferred that factors other than these metal contaminants may have a dominant role in affecting the condition. Cumulative effects of heavy metals and various types

of pollutants including organic compounds may have influence on the condition of oysters (Roper, Pridmore *et al.* 1991). Lowest condition of oysters at all the five estuaries was observed between January to April 2003. This lower condition may be a result of seasonal variations of the life cycle of oysters. Because the condition of oysters is greatly influenced by the reproductive cycle of the animal (Roper D.S, Pridmore R.D *et al.* 1991). The Pacific oysters in New Zealand coastal areas spawn between February to April (Peter 1990). Therefore the drop seen in condition of oysters, in this study between January to April could probably be due to the spawning event of the oysters. During this period the tissues contain a low level of lipid and glycogen with higher amount of water (Roper D.S, Pridmore R.D *et al.* 1991). This in turn result in a low dry flesh weight and observed as decreased condition index (Roper D.S, Pridmore R.D *et al.* 1991; Scanes 1993). Release of gametes also could contribute to the observed reduction of condition during the period of January to April. After spawning, the condition showed an increasing trend from May to October. The increase of the CI during the period of May to August may be the result of the autumn and winter “fattening” of the oysters (Roper, Pridmore *et al.* 1991; Scanes 1993). After this autumn and winter “fattening” further weight gain can be observed due to the ripening of the reproductive organs. The increase the weight of the soft tissues during pre spawning time of their life cycle may be the cause observed increase of the condition of the oysters from May to October. The basic pattern observed in the variation of the condition cycle of the oysters with relation to the reproductive cycle in the present study was similar to the pattern of variation of the condition observed by Roper Pridmore *et al* (1991), in the oysters of Manukau Harbour.

However, the fluctuations have observed from the basic pattern of condition during the period of May to October at all the five sites. Though this period represent the autumn and winter “fattening” of the oysters the fluctuations may be due to growth retardation as a result of an influence of an unfavourable environmental or biological factor or may be owing to some experimental error. This observed slow growth rate may be considered as the reflection of the local environmental factors. Hence no uniformity was observed in the fluctuations between the sites.

A number of reasons have been put forward as an attempt to explanation for the observed variations of the condition of the oysters at the river mouths of Mahurangi, Awaruku, Waiake, Taiorahi and Wairau in the present study. Observed environmental variabilities among the five river mouths were carefully considered to describe spatial variability of the condition of the oysters at the five sites. The condition of the environment reflects the condition of the bivalves because the reduction of CI is associated with physical stress of the animals due to the prevailing unfavourable factors of the environment (Lucas and Beninger 1985; Goldberg and Bertine 2000; Palmers 2000).

The higher condition of the oysters seen at the river mouth of Awaruku and Wairau ( $4.3 \mu\text{g/g} \pm 0.5$  and  $4.1 \mu\text{g/g} \pm 0.2$ ) respectively can be related to the physical nature of the inter tidal zone and the observed water quality differences of the localities. Oyster beds in these two river mouths have more or less flattened inter tidal zone with high algal growth contrasting to other river mouth habitats. It could therefore be expected that oysters may be submerged for longer periods in between tides. As a result oysters may have minimal exposure to unfavourable conditions such as desiccation and temperature fluctuations. This could therefore be a contributing factor to higher growth rate which in turn observed as higher condition of the oysters in the sites.

The condition of oysters at Waiake and Taiorahi has been the lowest among all the sites ( $2.88 \mu\text{g/g} \pm 0.30$  and  $3.06 \mu\text{g/g} \pm 0.30$  respectively). The sewer pumping station situated close to the oyster beds in Waiake river mouth may have occasional sewer outflow. The pollution such as micro-organisms, nutrients, and oxygen depleting organic matter from the sewer overflow may alter the environmental factors that could effect the growth of the oysters in that environment, and in turn effect the condition of the oysters living in that locality. Oysters also have the ability to retain faecal micro organisms specifically viruses (Donnison and Ross 1999). Therefore the possible micro organism contamination due to sewer outflow may have a negative impact on the condition of the oysters. This could probably be the reason for observed

lower condition in oysters at the river mouth of Waiake. However, it is necessary to carry out more control investigations to confirm this argument.

Sampled oysters at the river mouth of Taiorahi were attached to steep rock surfaces and the oyster beds were situated on higher level in the intertidal zone compared to Awaruku and Wairau. Hence these oyster beds are exposed for longer period of time between tides. Population density of these oyster beds was also seemed to be higher than that of oysters at Awaruku and Wairau. Because of high competition for the space and longer exposure to unfavourable conditions, may have contributed for the observed lower condition of oysters at the river mouth of Taiorahi compared to the river mouths of Awaruku and Wairau

#### **4.3 Summary**

Higher level of copper, lead and zinc was found in the oysters sampled at the river mouths of Waiake, Taiorahi and Wairau than the oysters sampled at the river mouths of Mahurangi and Awaruku. This represented the urbanised and industrialised land use of the catchments of Waiake, Taiorahi and Wairau. Cadmium concentration was higher in oysters at the river mouth of Mahurangi than the oysters of the other river mouth locations. The observed higher cadmium concentration in the oysters of the Mahurangi river mouth was possibly due to heavy use of cadmium fertilizers on the pasturelands of the catchments of Mahurangi river. Heavy metal content in the sediments reflected the pollution status of the coastal environments of the river mouths. Rivers with heavily urbanised and industrialised catchments contained more heavy metal contaminants in the sediments. Condition of the oysters at the five river mouths showed a clear relationship with the seasonal changes of the reproductive cycle of the oysters. Lowest condition was observed in the oysters during the months of December to April. During the spawning period of the life cycle, body weight decreased due to release of the gametes resulting lower condition of the oysters. Spawning time was greatly influenced by number of environmental factors such as heavy metal concentration, temperature and salinity of the water.

The condition of the oysters was reported high at the river mouths of Wairau. The suggested reason was the presence of high amount of particulate matter which acts as a food source of the oysters.

## **Chapter 5**

### **Conclusion**

The observed variation of cadmium, copper, lead and zinc concentrations in the oyster tissues and the sediments at the studied five river mouths on the north eastern coast of Auckland indicated the relationship between the land use of the catchments of the rivers and the heavy metal pollution of the coastal waters. The catchment areas of these studied rivers have been undergoing rapid changes due to urban and industrial development occurring during last few years.

The observed higher concentrations of copper and zinc in Mahurangi, Awaruku Waiake, Taiorahi and Wairau river mouths and cadmium in Mahurangi river mouth in oysters and the heavy metal concentration in sediments of the studied river mouths reflects the impact of coastal pollution on shellfishes, which are an important source of contamination in the human diet (Okazaki and Panietz 1981). Soil erosion, sewage outflow and some natural conditions such as precipitation amplify the copper contamination in urban river waters (Butler and Timperley 1996).

The observed higher concentration of copper and zinc in oyster tissues from all the estuaries indicates the prevalence of copper and zinc contamination in north eastern coast of Auckland. Higher cadmium concentration levels found in the oysters sampled from the river mouth of Mahurangi reflects the effects of heavy use of cadmium fertilizers on the surrounding farm lands. The detected higher concentration of copper and zinc in oysters and sediments from all the estuaries

exceeded the prescribed limits for the human consumption of shellfishes (Australia New Zealand Food Standard Code 2002). This indicates the prevalence of heavy metal contamination in the north eastern coast of Auckland.

Results of this study showed a relationship between the land use of the catchments of the river mouths of Mahurangi, Awaruku Waiake, Taiorahi and Wairau and concentration of heavy metals in oyster tissues. Therefore Pacific oysters have ability to indicate environmental heavy metal levels. Thus the Pacific oysters can be used as bioindicators to detect aquatic the heavy metal pollution in shallow coastal areas.

Though the catchment of Mahurangi stream comprises of less urbanised and industrialised land use, the estuary contained higher level of cadmium than the other river mouth environments. This indicates that not only the catchments with industrial land use, but also farmlands such as catchment of Mahurangi could contribute heavy metal pollution of coastal environment, especially metals such as cadmium.

Lack of proper management of the lands of the river catchments may create a potential health risk if shellfishes such as oysters are consumed. Though the urban coastal areas of New Zealand are protected by the Water and Soil Conservation Act (1967), there are numerous uncontrolled outlets carrying contaminants from terrestrial areas, such as faecal pollutants particularly at sites where there is discharge from treated sewage, treated meat-processing wastewater or inputs by dairy farms, surface runoff of pasture lands and stormwater discharge (Penny 1984; Donnison and Ross 1999).

Shellfish aquaculture is often associated with nutrient rich waters to ensure adequate algal productivity. But this study has shown coastal areas with potential to have anthropogenic input of pollutants are great threat to the aquaculture industry (Okazaki and Panietz 1981). Hence heavy metal pollution could become a significant problem in coastal environment especially areas with oyster farms. Therefore heavy metal contamination of shellfishes is

difficult to avoid. To minimise coastal pollution, control measures have to be excised at the source of the origin of discharge. For example lead contamination in the environment was successfully achieved by the controlling the use of leaded petrol.

Industrial discharge and heavy use of agricultural contaminants such as fertilizers and pesticides should be controlled as a remedy to minimise coastal pollution. Sub-lethal base concentration levels of agricultural contaminants in the aquatic habitats can be maintained by reducing the use of fertilizers and pesticides on the catchment farmlands of the rivers. To implement this method it requires reliable toxicity information supported by well documented field studies. Damage caused to the aquatic habitats by industrial discharges can be minimised by treating effluents to remove pollutants before they are discharged. However, this method is not practical due to its high cost.

Observed seasonal variations of the condition of oysters might be attributed to a variety of factors including temperature, salinity, food availability, metabolic activities and was strongly influenced by seasonal changes in reproductive cycle.

*Crassostrea gigas* showed the lowest condition during the period of late summer and early autumn. The spawning times of oysters at different study locations were not similar to each other. This difference in the spawning time may be due to the effect of environmental factors, such as temperature which has direct influence on the spawning time. Unfortunately, there were no available data in the temperature variations of the water in these studied river mouths. It is clear that much additional work will be required before complete understanding of the difference in the spawning time of oysters at different river mouths.

The observed higher condition, despite higher levels of copper and zinc is considered to have resulted from the environmental factors of the Wairau river mouth. It is hoped that this work will stimulate further work to investigate the factors affecting the condition of the oysters at the Wairau river mouth.



This study will help identify the extent of pollution of various coastal areas along the north-eastern coastal areas of Auckland. This, study will also enhance our knowledge about the rates and amounts of bio-accumulation of cadmium, chromium, copper lead and zinc in oyster tissues, and will promote understanding the general ecology of oysters living in river mouths along North Eastern coastal areas in Auckland.

Finally, the variations in cadmium, chromium, copper, lead and zinc concentrations in the studied coastal areas in north eastern coast of Auckland will provide valuable information to detect unknown areas of heavy metal contamination.

## Appendix – 1: Results of t- test of AGAL – 10 and the TAE certified values

### Two-Sample T-Test and CI - Cd

Sample	N	Mean	StDev	SE Mean
1	5	9.110	0.650	0.29
2	12	9.090	0.368	0.11

Difference =  $\mu$  (1) -  $\mu$  (2)  
Estimate for difference: 0.020000  
95% CI for difference: (-0.775575, 0.815575)  
T-Test of difference = 0 (vs not =): T-Value = 0.06 P-Value = 0.951 DF = 5

### Two-Sample T-Test and CI - Cu

Sample	N	Mean	StDev	SE Mean
1	5	23.51	1.63	0.73
2	12	24.52	1.78	0.51

Difference =  $\mu$  (1) -  $\mu$  (2)  
Estimate for difference: -1.01000  
95% CI for difference: (-3.06548, 1.04548)  
T-Test of difference = 0 (vs not =): T-Value = -1.13 P-Value = 0.290 DF = 8

### Two-Sample T-Test and CI - Pb

Sample	N	Mean	StDev	SE Mean
1	5	42.34	5.24	2.3
2	12	40.11	1.14	0.33

Difference =  $\mu$  (1) -  $\mu$  (2)  
Estimate for difference: 2.23000  
95% CI for difference: (-4.33983, 8.79983)  
T-Test of difference = 0 (vs not =): T-Value = 0.94 P-Value = 0.399 DF = 4

### Two-Sample T-Test and CI - Zn

Sample	N	Mean	StDev	SE Mean
1	5	57.12	3.08	1.4
2	12	54.13	6.72	1.9

Difference =  $\mu$  (1) -  $\mu$  (2)  
Estimate for difference: 2.99000  
95% CI for difference: (-2.11081, 8.09081)  
T-Test of difference = 0 (vs not =): T-Value = 1.26 P-Value = 0.229 DF = 14

## References

- Abbe, G. R., G. F. Riedel, et al. (2000). "Factors that influence the accumulation of copper and cadmium by transplanted eastern oysters (*Crassostrea virginica*) in the Patuxent river, Maryland." Marine Environmental Research **49**(4): 377 - 396.
- Australia New Zealand Food Standard Code (2002). "Contaminants and natural toxicants." Food Standard Code: 251.
- Boyden, C. R. (1974). "Trace element Content and Body Size in Molluscs." Nature **251**: 311-314.
- Brown, J. R. and E. B. Hartwick (1988). "Influences of Temperature, salinity and food upon suspended culture of the Pacific oyster, *Crassostrea gigas*. 11. Condition index and survival." Aquaculture **70**(3): 253-267.
- Bunce, N. J. (1994). Environmental Chemistry. Winnipeg, Wuerz Publishing Ltd.
- Burden, F. R., Ed. (2002). Environmental Monitoring Handbook. Soils and Sediments. New York, McGraw - Hill.
- Butler, C. A. and M. Timperley (1996). "Fertilised farmland as a source of cadmium in oysters." The Science of The Total Environment **181**: 31-44.
- Clark, R. (1997). Marine Pollution. Oxford, Clarendon press.
- Cunningham, P. A. and M. R. Tripp (1973). "Accumulation and depuration of mercury in the American oyster *Crassostrea virginica*." Marine Biology **20**: 14-19.
- Davenport, J. and X. Chen (1987). "A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.)." Journal of Molluscan Studies **53**: 293 - 297.

Denton, G. R. W. and C. Burdon-Jones (1981). "Influence of Temperature and salinity on the uptake, distribution and depuration of mercury, cadmium and lead by the black-lip oyster." Marine Biology **64**: 317-326.

Donnison, A. and C. Ross (1999). "Animal and Human Faecal Pollution of New Zealand rivers." Wastewater: 29-32.

Edwards, J. W., K. S. Edyvane, et al. (2001). "Metal level in seston and marine fish flesh near industrial and metropolitan centres in South Australia." Marine Pollution Bulletin **42**(5): 389-396.

Fisher, N. S. and J. R. Reinfelder (1995). The Trophic transfer of Metals in Marine Systems. Chichester, John Wiley and Sons.

Forstner, U. and G. T. W. Wittmann (1979). Metal Pollution in the Aquatic Environment. Berlin, Springer-Verlag.

Frazier, J. M. (1976). "The dynamics of metals in the American oyster, *Crassostrea Virginica*. II. Environmental effects." Chesapeake Science **17**(3): 188 - 197.

Fresenius, W., K. E. Quentin, et al. (1988). Water Analysis. Berlin, Springer - Verlag.

Galtsoff, P. S. (1964). The American Oyster. Washington, Unites States Government Printing Office.

Gaw, S. (1997). Interactions of Copper, Lead and Zinc with Stormwater Impacted Sediments in Puhinui Stream, Manukau City, New Zealand, University of Auckland.

George, S. G. (1983a). "Heavy Metal Detoxification in *Mytilus edulis*. Composition of Cd - containing kidney granules (tertiary lysosomes)." Comparative Biochemistry and Physiology **76C**: 59-65.

Glasby, G. P., P. Stoffers, et al. (1988). "Heavy metal pollution in Manukau and Waitamata harbours, New Zealand." New Zealand Journal of Marine and Fresh Water **22**: 595-611.

Goldberg, E. D. and K. K. Bertine (2000). "Beyond the Mussel Watch - new directions for monitoring marine pollution." The Science of The Total Environment **247**: 165 - 174.

Gorman, M. (1952). Environmental Hazards, Marine Pollution. Santa-Barbara, California, ABC-CLIO.

Gorman, M. (1993). Environmental hazards-Marine pollution. Santa Barbara, California, ABC - CLIO.

Gosling, E. (2003). Bivalve Molluscs. Bodmin, Blackwell Publishing.

Gulliver, K. (2001). The assessment of the Effects Of The Pollutants Attributed To Urbanisation Upon The Aquatic Environment At The Long Bay Okura Marine Reserve North Shore New Zealand. Faculty of Applied Science. Auckland, Auckland University of Technology: 138.

Han, B. C., W. L. Jeng, et al. (1998). "Estimation of target hazard quotients and potential health risks for metals by consumption of seafood in Taiwan." Archives of Environmental Contamination and Toxicology **35**: 711 - 720.

Hickey, B. A. (1992). *Perna canaliculus* As A Biomonitor Of Water Quality: Metal Accumulation And Health Indicators. Auckland, University of Auckland.

Huang, K. M. and S. Lin (2003). "Consequences and implications of heavy metal spatial variations in sediments of the Keelung river drainage basin, Taiwan." Chemosphere **53**(9): 113 - 1121.

Huanxin, W., Z. Lejun, et al. (2000). "Bioaccumulation of heavy metals in oyster (*Crassostrea virginica*) tissue and shell." Environmental Geology **39**(11): 1216-1226.

Jackim, E., G. Morrison, et al. (1977). "Effects of environmental factors on radiocadmium uptake by four species of marine bivalves." Marine Biology **40**: 303 - 308.

Jamil, K. (2001). Bioindicators and Biomarkers of Environmental Pollution and Risk Assessment. Enfield, USA, Science Publishers Inc.

Jones, G. B., P. Mercurio, et al. (2000). "Zinc in fish, crabs, oysters and mangrove flora and fauna from Cleveland Bay." Marine Pollution Bulletin **41**(7-12): 345-352.

Kahraman, N., I. Olmez, et al. (1976). Measurement, Detection, and Control of Environmental Pollutants. Vienna, International Atomic Energy Agency.

Kennish, M. J. (1992). Ecology of Estuaries: Anthropogenic Effects. London, CRC Press.

Kennish, M. J. (1997). Practical handbook of Estuarine and Marine Pollution. Boca Raton, CRC Press Inc.

Ketchum, B. H. (1980). Marine Industrial Pollution, in Oceanography: The Past. New York, Springer-Verlag.

Kim, Y., E. N. Powell, et al. (2001). "the Geographic Distribution of Population Health and Contaminant Body Burden in Gulf of Mexico Oysters." Archives of Environmental Contamination and Toxicology **4**: 30-46.

Klein, M. A. (1994). Indicators of Assessment of the environmental Impact of a Landfil. Auckland, University of Auckland.

Krogh, M. and P. Scanes (1996). "Organochlorine compound and trace metal contaminants in fish near Sydney's ocean outfalls." Marine Pollution Bulletin **33**(7 - 12): 213 - 225.

Kushel, G. and M. Timperley (1999). The Effect of StormWater and Transport on Urban Streams and Estuaries. Water and Atmosphere. **7**: 22-25.

Legorburu, I. and L. Canton (1991). "Heavy metal concentration in sediments from Pasajes Harbour, Spain." Marine Pollution Bulletin **22**: 207 - 209.

Lenihan, J. and W. W. Fletcher (1978). Environment and man. Glasgow and London, Blackie.

Louie, H. (1994). Certificate of analysis for reference material AGAL - 10, Hawkesbury River sediment, Australian Government Analytical Laboratories (AGAL), New South Wales.

Lucas, A. and P. G. Beninger (1985). "The use of physiological condition indices in marine bivalve aquaculture." Aquaculture **44**: 187 - 200.

Luoma, S. N. (1989). "Can we determine the biological availability of sediment - bound trace elements?" Hydrobiologia **176/177**: 379 - 396.

Manahan, S. E. (1993). Fundamentals of Environmental Chemistry. Boca Raton, Lewis Publishers.

Mance, G. (1987). Pollution Threat of Heavy Metals in Aquatic Environment. London and New York, Elsevier Applied Sciences.

Mance, G. (1987). Pollution Threat of Heavy Metals in Aquatic Environments. London, New York, Elsevier Applied Science.

Martincic, D., Z. Kwokal, et al. (1990). "Distribution of lead, zinc, cadmium and copper between different site fractions of sediments 1. The Limski Kanal (North Adriatic Sea)." Science of the Total Environment **95**: 201 - 215.

Muhammad, S. (1992). Toxic metal chemistry in marine environments. New York, Marcel Dekker Inc.

Nielsen, S. A. and A. Nathan (1975). "Heavy metal levels in New Zealand Molluscs." New Zealand Journal of Marine and Fresh Water Research **9**(4): 467 - 481.

Nriagu, J. O. (1989). "A global assessment of natural sources of atmospheric trace elements." Nature **338**: 47-49.

O'Connor, T. (1998). Chemical Contamination in Oysters and Mussels, National Oceanic and Atmospheric Administration. **2002**.

Okazaki, R. K. and M. H. Panietz (1981). "Depuration of twelve trace metals in tissues of the oysters *Crassostrea gigas* and *C. virginica*." Marine Biology **63**: 113 - 120.

Olson, T. A. and F. J. Burgess, Eds. (1967). Pollution and Marine Ecology. New York, Interscience Publishers.

Osuna, F. P., M. G. F. Espericueta, et al. (1995). "Trace metal concentrations in relation to season and gonadal maturation in the oyster *Crassostrea iridescens*." Marine Environmental Research **40**(1): 19 - 31.

Palmer, H. E. (2000). Monitoring Pacific oyster condition: Correlations with environmental variations. Department of Marine Science. Dunedin, University of Otago.

Patin, S. A. (1982). Pollution and biological resources of the oceans. London, Butterworth Scientific.

Peerzada, N. and W. Rohoza (1989). "Some heavy metals in sediments from Darwin Harbour, Australia." Marine Pollution Bulletin **20**: 91-92.

Penny, S. (1984). "The effects of fine sediment and heavy metal inputs to the coastal environment: A literature review." New Zealand Environment **43**: 12-16.



Peter, K. (1990). Growth and Reproduction of Pacific Oysters. School of Biological Sciences. Auckland, University of Auckland.

Phillips, D. J. H. (1976). "The common mussel *Mytillus edulis* as an indicator of pollution by zinc, cadmium, lead and copper .I. Effects of environmental variables on uptake of metals." Marine Biology **38**: 59 - 69.

Phillips, D. J. H. (1977). "The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments." Environmental Pollution **13**: 281 - 317.

Phillips, D. J. H. and P. S. Rainbow (1992). Biomonitoring of Trace Aquatic Contaminants. London, Elsevier Applied Science.

Phillips, D. J. H. and W. W. S. Yim (1981). "A comparative evaluation of oysters, mussels and sediments as indicators of trace metals in Hong Kong waters." Marine ecological Progress Series **6**: 285-293.

Pridmore, R. D., D. Roper, et al. (1990). "Variation in Composition and Condition of the Pacific Oyster *Crassostrea gigas* Along a Pollution Gradient in Manukau Harbour, New Zealand." Marine Environmental Research **30**: 163-177.

Prudente, M. S., H. Ichihashi, et al. (1994). "Heavy metal concentrations in sediments from Manila Bay, Philippines and inflowing rivers." Environmental Pollution **86**: 83 - 88.

Rainbow, P. S. (1995). "Biomonitoring of heavy metal availability in the marine environment." Marine Pollution Bulletin **31**(4 -12): 183 -192.

Rebelo, M. d. F., M. C. R. d. Amaral, et al. (2003). High Zn and Cd accumulation in the oyster *Crassostrea rhizophorae*, and its relevance as a sentinel species, Marine Pollution Bulletin. **2003**.

Roberts, G. S. (2000). North Shore City Council Project Care. Auckland, North Shore City Council.

Roesejadi, G. (1980). "The significance of low molecularweight, metallothionein- like proteins in marine invertebrates: current status." Marine Environmental Research **4**: 167-181.

Roper D.S, Pridmore R.D, et al. (1991). "Pollution Related Differences in the Condition Cycles of Pacific Oyster *Crassostrea gigas* from Manukau Harbour, New Zealand." Marine Environmental Research **31**: 197-214.

Roper, D. S., R. D. Pridmore, et al. (1991). "Pollution Related Differences in the Condition Cycles of Pacific Oyster *Crassostrea gigas* from Manukau Harbour, New Zealand." Marine Environmental Research **31**: 197-214.

Samoiloff, M. R. (1989). Aquatic Toxicology and Water Quality Management. New York, John Willey and sons.

Saunders, J. and B. Creese (2000). Baseline Monitoring of Long Bay-Okura Marine Reserve. Auckland, Leigh Marine Laboratory.

Scanes, P. (1993). "Trace metal uptake in cockles *Anadara trapezium* from lake Macquarie, New South Wales." Marine ecological Progress Series **102**: 135 - 142.

Shulkin, V. M., B. J. Presley, et al. (2003). "Metal concentration in mussel *Crenomytilus grayanus* and oyster *Crassostrea gigas* in relation to contamination of ambient sediments." Environment International **29**(4): 493-502.

Silva, C. A. R., P. S. Rainbow, et al. (2001). "Biomonitoring of Trace Metal Contamination In The Potengi Estuary, Natal (Brazil), Using The Oyster *Crassostrea Rhizophorae*, A Local Food Source." Water research **35**(17): 4071 - 4078.

Snelder, T. and S. Trueman (1995). The Environmental Impacts of Urban Stormwater Runoff. Auckland, Auckland Regional Council, New Zealand.

Soares, H. M. V. M., R. A. R. Boaventura, et al. (1999). "Sediments as monitors of heavy metal contamination in the Ave river basin (Portugal) : multivariate analysis of data." Environmental Pollution **105**(3): 311 - 323.

Soule, D. F. and G. S. Kleppel (1987). Marine Organisms as Indicators. New York, Springer.

Steven, B. (1999). Long Bay Structure Plan. Auckland, North Shore City Council: 50.

Taylor, M. P., S. Lunn, et al. (2003). Heavy metal contamination of Mt Isa - Implications for the ecosystem. In Search Of Sustainability, Australia.

Technical Publication (2001). Manukau Harbour Shellfish Quality Survey. Auckland, Auckland Regional Council: 47.

Thomas, W. A. (1975). Indicators of environmental quality. New York, Plenum Publishing Corporation.

Timperley, M. (2000). "StormWater And Transport Effects on urban Aquatic Ecosystems." NIWA Publication - S.W.A.T. update **1**(2): 8.

Timperly, M. (2001). "StormWater And Transport Effects." NIWA Publication - S.W.A.T. update **2**(1): 6.

Tinsley, L. J. (1979). Chemical Concepts in Pollutant Behaviour. New York, Chichester, Brisbane, Toronto, John Wiley and Sons.

van Roon, M. (1999). Availability, Toxicity and uptake of Heavy Metals by Marine Invertebrates. Auckland, University of Auckland.

Vernberg, W. B., F. Thurnberg, et al., Eds. (1979). Marine Pollution :Functional Response. The Use of Bivalve Molluscs in Heavy Metal Pollution Research. New York, Academic Press.

Viarengo, V. (1985). "Biochemical Effects of Trace Metals." Marine Pollution Bulletin **16**(4): 153 - 158.

Wood, J. M. (1974). "Biological Cycles for Toxic Elements in the Environment." Science(183).

Young, C. M. (1960). Oysters. London, Collins.

Zaroogian, G. E. (1980). "Crassostrea virginica as an indicator of cadmium pollution." Marine Biology **58**: 275 - 284.

Zaroogian, G. E., G. Morrison, et al. (1979). "Crassostrea virginica as an indicator of lead pollution." Marine Biology **52**: 189 - 196.