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METAL CONTAMINATION AND ANTIOXIDANT RESPONSES OF *MYTILUS GALLOPROVINCIALIS* ALONG THE WEST COAST OF THE CAPE PENINSULA, SOUTH AFRICA

by

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DECLARATION

I, Conrad Sparks, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed Date

ABSTRACT

Metals are prevalent in the marine environment and come from various sources that are of both natural and anthropogenic origins. High concentrations of metals can have negative impacts on marine organisms and hence knowledge about the levels of contamination is important. However, knowledge about the levels of metal contamination in southern Africa and in particular, Cape Town, is sparse and there is a need to ascertain the levels and effects of metals in both the environment and biota. Metal bioaccumulation in *Mytilus galloprovincialis* sampled from 1985 to 2008 during the Mussel Watch Programme (MWP) was analysed. Analysis of MWP mussels from the west coast of the Cape Peninsula, South Africa showed that metal contamination varied significantly between sites and seasons. The mean order of combined metal concentrations for the study period was Zn>Fe>Cd>Cu>Mn>Hg.

The concentrations of metals in the water, sediment and mussels (*Mytilus galloprovincialis)* were measured along the west coast of the Cape Peninsula from autumn 2010 to autumn 2011. Sampling took place at Scarborough, Hout Bay, Green Point, Milnerton and Bloubergstrand. The samples were digested using nitric acid and analysed using an Inductively-Coupled Plasma-Mass Spectrophotometer (ICP-MS). The results of the water and sediment analysis showed that metal contamination was lowest in Scarborough and highest in Hout Bay. Seasonally, pollution loads were highest in autumn 2011 and lowest in autumn 2010. The high contamination levels reported in the sediment suggested that localised contamination from anthropogenic sources as well as natural weathering were responsible for the high concentrations of metals reported. The Pollution Load Index of metals in the sediment showed that the order of polluted sites were Hout Bay > Bloubergstrand > Green Point > Milnerton > Scarborough. The bioaccumulation of metals in *M. galloprovincialis* showed that most metals were significantly lowest at Scarborough and that metals in mussels differed significantly between seasons, with winter 2010 showing the highest significant difference. The efficiency of metal accumulation was measured using the Biosediment Accumulation Factor (BSAF). The results showed that the BSAF was highest for Cd, Pb, Zn and Cu, with the lowest BSAF reported for Fe and Mn. These results indicated that metals were bioavailable for uptake and suggested that *M. galloprovincialis* accumulated Cd, Pb and Zn at higher rates than

the other metals sampled, and could support the notion that these metals be ideally suited as tools for biomonitoring.

A laboratory experiment was done to investigate correlations between the concentrations of Cu in *M. galloprovincialis* and antioxidant responses caused by potential stress induced by Cu exposure. The results of the experiment indicated that copper accumulated in *M. galloprovincialis* during a 21-day exposure period. Mussels exposed to low dosages (40 µg/L) of Cu resulted in a 4-fold increase in Cu bioaccumulation in its tissue, whereas mussels exposed to high dosages (100 µg/L) of Cu resulted in a 10-fold increase in Cu bioaccumulation in its tissue. The potential of oxidative stress as a biomarker was investigated using a battery of antioxidant biomarkers in the mussels exposed to Cu. Total antioxidant capacity was measured using FRAP and ORAC, enzyme activity was determined using CAT, SOD and GSH and lipid peroxidation was determined using TBARS and CDs. The results showed that *M. galloprovincialis* exposed to high dosages of Cu had significantly higher antioxidant activities, assumed as a response to the high dosages of Cu that the organisms were exposed to. For GSH, ORAC and TBARS, the antioxidant activities by day 21 were significantly higher than at the start of the experiment. There were significant differences between exposure groups and times, but these differences did not change consistently over time. The results of this chapter suggested that antioxidant responses could be considered as biomarkers of toxicity and it is recommended that future research considers mixtures of contaminants that reflect the metals in the natural environment.

Antioxidant responses in *M. galloprovincialis* were determined from samples collected at Scarborough, Hout Bay, Green Point, Milnerton and Bloubergstrand. The results showed variable antioxidant responses at the respective sites, making interpretation of the antioxidant biomarker activities difficult. The results suggest that, based on antioxidant enzyme activity, Milnerton be considered a relatively polluted site. The antioxidant capacity biomarkers suggested that Green Point, Milnerton and Bloubergstrand be considered relatively polluted. The lipid peroxidation responses suggested that only Milnerton and Bloubergstrand be considered relatively polluted. However, it is noted that there could have been other factors that could have caused oxidative stress, including naturally induced activities such wave action, desiccation,

lack of food, etc. The oxidative stress results should therefore be considered with caution since all the sites sampled are not considered to be polluted when compared to international standards. Based on these findings, it was recommended that Milnerton and Bloubergstrand could be considered relatively polluted, Hout Bay and Green point mildly relatively polluted and Scarborough, unpolluted. It was concluded that antioxidant responses was an appropriate biomarker of stress but that more environmental parameters be considered when interpreting the antioxidant responses.

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DEDICATION

This thesis is dedicated to

Claudine, Damian and Liam

Thank you for your love, encouragement and support.

I love you

My parents

Jean Sparks

(1956 – 1977)

and

Archibald Paulsen

(1948-1980)

Although I never had the chance to spend time with you, I thank God that, because of you, I am.

"Not now, but in the coming years, it may be in a better land, we'll read the meaning of our tears, and there, sometime we'll understand"

(Hymn 422 of NAC Hymnal)

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
6-HD	6-hydroxydopamine
AA	L-Ascorbic acid
AAE	Ascorbic Acid Equivalents
AAPH	2,2'azobis (2-amidinopropane) dihydrochloride
ANOSIM	Analysis of similarity
ANOVA	Analysis of Variation
BHT	Butylated hydroxytoluene
BSAF	Biosediment accumulation factor
C. meridionalis	*Choromytilus meridionalis*
Cape Town	City of Cape Town
CAT	Catalyse
CSQG	Canadian sediment quality guidelines
CD	Conjugated diene
CF	Contamination factor
CPUT	Cape Peninsula University of Technology
$CuSO_4$	Copper sulphate

DAFF	Department of Agriculture, Forestry and Fisheries
DDT	Dichlorodiphenyltrichloroethane
DEAT	Department of Environmental Affairs and Tourism
DETAPAC	Diethylenetriaminepentaacetic acid
DTNB	5,5'-dithiobis (2-nitrobenzoic acid)
DWAF	Department of Water and Forestry
EBC	Eastern Boundary Current
EBM	Ecosystem Based Management
EDTA	Ethylenediaminetetra-acetic acid
ERA	Ecological Risk Assessments
$FeCl_3$	Iron (III) chloride
FRAP	Ferric reducing antioxidant power
GDP	Gross domestic product
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
GSX-PX	Glutathione peroxidase
H_2O_2	Hydrogen peroxide

H_3PO_4	Ortho-phosphoric acid
HCl	Hydrochloric acid
HSD	Honest Significant Difference
KCl	Potassium chloride
KPO_4	Potassium phosphate
LPO	Lipid peroxidation
M. galloprovincialis	*Mytilus galloprovincialis*
M2VP	1-methyl-2-vinylpyridinium trifluoromethanesulphonate
MANOVA	Multivariate analysis of variation
MDA	Malondialdehyde
MDS	Multi-dimensional scaling
MEC	Measured Environmental Concentration
MT	metallothioneins
MWP	Mussel Watch Programme
NaCl	Sodium chloride
NADPH	nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
$NaPO_4$	Sodium di-hydrogen orthophosphate mono hydrate

ND	Not detected
ORAC	Oxygen radical absorbance capacity
P. perna	*Perna perna*
PCA	Perchloric acid
PCB	Polychlorinated biphenyl
PEC	Predicted Environment Concentration
PLI	Pollution Load Index
PNEC	Predicted No Effect Concentrations
PRIMER	Plymouth Routines in Multivariate Ecological Research
ROS	Reactive oxygen species
SABS	South African Bureau of Standards
SD	Standard deviation
SE	Standard error
SIMPER	Similarity percentages
SOD	Superoxide dismutase
SWCB	South Western Coastal Belt
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid

TBT	Tributyltin
TE	Trolox equivalents
TPTZ	2,4,6-Tripyridyl-s-triazine
Trolox	6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid

Chapter 1

GENERAL INTRODUCTION

1.1. Status of the marine ecosystem

The sea was once regarded a “bottomless pit” into which all waste was discarded without consideration for effects the waste might have had on the ecosystem (Diamant & Westernhagen, 1999). According to Connell *et al.* (1999), the number of chemicals in routine use by humans is vast and it has been estimated that more than 70 000 chemicals are commonly used globally. From such chemicals a further greater number of chemical wastes are produced, some with end-products that have disastrous effects on both organisms and ecosystems. The notion therefore that the sea is a bottomless pit has been rejected due to extinctions and extirpations of major populations and even ecosystems (Connell *et al.,* 1999).

Only in the past 50 years have serious attempts been made to reduce the destruction of marine ecosystems. The 1962 publication of *Silent Spring* by Rachel Carson emphasized the dangers of pesticides and made the public aware of the negative effects of pesticides on the environment, in particular humans and wildlife (Carson, 1962). There have been a number of environmental events that further justified the need for action to protect the environment (the deleterious effects of DDT, Minimata disease in Japan) (Connell *et al.*, 1999; Wright & Welbourn, 2002). By the 1970’s environmental problems were able to be broadcast via television across the globe and this elevated the expressive concerns of many people. In particular, the environmental movement used political pressure to address issues concerning the destruction of the environment. The outcomes of these actions were laws and regulations that served as tools for environmental protection (Wright & Welbourn, 2002). The protection of the environment is important as it ensures sustainable biodiversity structure and function.

According the Beaumont *et al.* (2007), marine biodiversity provides ecosystem goods and services and the values of these are dependent on the state of the whole ecosystem. Ecosystem services include provisioning (*e.g.* medicinal plants and firewood), regulatory (*e.g.* water purification and regulation), supporting (*e.g.* soil

retention and formation), and cultural services (the use of nature for cultural and spiritual purposes) (Egoh *et al.*, 2009). Hence, to derive full economic (and ecological) value for the marine ecosystem, it is beneficial to have a system that is in a pristine state. The effect of over-harvesting of resources is also a major constituent of ecosystem destruction as fishing efforts could cause imbalances of species interactions (such as predator-prey relationships) (Coleman & Williams, 2002). Even though the wide use of ecosystem services to argue for biodiversity conservation is known, there is paucity of information about how different aspects of biodiversity relate to ecosystem services and to what extent conserving biodiversity will ensure provision of those services (Egoh *et al.*, 2009). As a result, conserving biodiversity integrity and how ecosystem services are managed might require different approaches. Further impacting the (marine) ecosystem is pollution that has negative effects and consequences on the marine ecosystem. An understanding of pollution and its relation to toxicology is important as it provides an assessment of the status of marine environments (Wright & Welbourn, 2002). The status of marine environments in turn can provide further value to ecosystem goods and services.

Studies on marine pollution and the effects thereof have been at the forefront of marine research in the last decade (O'Donoghue & Marshall, 2003), mainly due to increased levels of contaminants in the marine environment caused by anthropogenic activities (Schlenk, 1999; Cajaraville *et al.*, 2000). Exposures to contaminants result in stress in organisms. The sources of stress-inducing agents in the marine environment are urbanization and industrial development along coastal areas that introduces diverse quantities of organic and inorganic man-made chemical compounds (Bresler *et al.*, 1999). The stress can either be the result of chronic (disturbances at low levels over a long period) or acute (a disturbance that is discrete, usually a large event) disturbances (Crowe *et al.*, 2000). Knowledge about the type of pollutant is important as it will determine the nature of the management of the marine ecosystem concerned.

The management of marine ecosystems (including South Africa) is now moving towards Ecosystem Based Management (EBM) (Beaumont *et al.*, 2007; Curtin & Prellezo, 2010). In an attempt to control and regulate the input of detrimental substances into the sea, the London Dumping Convention was signed in 1972

(Diamant & Westernhagen, 1999). In South Africa, protection of marine environments is promulgated in legislative Acts such as the Marine Pollution Act (6 of 1981), Environment Conservation Act (Act 73 of 1989), Dumping at Sea Control Act (36 of 1998), National Environmental Management Act (107 of 1998), National Environmental Management: Protected Areas Amendment Act (31 of 2004), National Environmental Management: Biodiversity Act (10 of 2004), National Parks Act (57 of 1976), Forests Act (122 of 1984), National Forests Act (84 of 1998), Marine Living Resources Act (18 of 1998), Mountain Catchment Areas Act (63 of 1970), World Heritage Convention Act (49 of 1999); National Heritage Resources Act (25 of 1999), and Sea Birds and Seals Protection Act (46 of 1973). The Acts are fragmented and incongruently manage various aspects of the marine ecosystem (Glavovic, 2006).

1.2. South Africa's marine ecosystem

The South African coast is divided into three biogeographical provinces: the cold temperate west coast, warm temperate south coast and subtropical east coast (Emanuel *et al.*, 1992). The coastal zone is not only important economically, but arguably more important due to its ecological significance. The main reason for this is that the coastal zone is the area where open sea systems meet the terrestrial system. As a result, the coastal zone is highly dynamic and it is this dynamic nature that has not only high ecological value, but also has high economic value as well.

Within the coastal zone, the littoral zone in particular is a complex geomorphological system. According to Dardis and Grindley (1988), there are five main reasons for the complexity: physical regime, sediment supply, climate, tectonic setting and sea level. Of these, the physical regime is the focal point, in particular in the southern African context given the nature of the coastline and adjacent oceanography. The coast is subject to high wave energy and the effect of this energy results in the coastal zone having considerable physiographic heterogeneity. This in turn affects the structure of the biotic communities on rocky shores. The fundamental outcome of these dynamic features and events are the rocky shore, sandy beaches and mixed sand-rock shores of southern Africa that defines the important ecological heterogeneity features.

The South African coastline has the cold Benguela Current, an Eastern Boundary Current (EBC), moving equatorward on the west coast of the continent and the warm Agulhas Current flowing poleward along the east coast (Shannon, 1985). Although EBC's make up only 1% of the world's ocean, they account for 5% of global marine primary production and as much as 15% of global fish catches (Blanchette *et al.*, 2009). The sources of the Benguela Current include Indian and South Atlantic subtropical thermocline water, saline, low-oxygen tropical Atlantic water, and cooler, fresher deeper water (Shannon, 1985; Shannon & Nelson, 1986). Furthermore, the cold Benguela Current is one of the most productive ecosystems in the world (Cushing, 1969; Carr, 2002) with the lowest species richness (Blanchette *et al.*, 2009). The high ecological productivity has resulted in the Benguela System yielding very high fishery recruitment. This applies not only to the open ocean, but also to the coastal system. According to Blanchette *et al.* (2009), primary producers and filter feeders (mainly barnacles and mussels) occupy well over 60% of primary space. In this system, it is the only case where filter-feeders occupy the largest proportion of space (approximately 65%) and are more abundant than primary producers. Despite this high production, most intertidal regions do not support large commercial fisheries and harvest pressure is generally low (Blanchette *et al.*, 2009). This situation is however not uniform throughout South Africa, with certain intertidal areas being under severe harvesting pressure (such as the Eastern Cape) and those coastal areas are hence responsible for many fisheries sectors (in particular subsistence and small-scale commercial) in the region that are of social and economic significance (Sowman, 2006).

The high yield from the Benguela Current has resulted in high human population densities along the coast. Cape Town is one of the major coastal cities in the country and is rich in coastal marine resources (Sowman, 2006). Many of the resources are harvested for human consumption and therefore play an important role in the economy of the region and mussels are one such resource (Harris *et al.*, 2002; Sowman, 2006). Because mussels are sentinel organisms, their health is dependent on the status of the ambient marine environment. This has further implications for human health as the mussels are harvested for human consumption and polluted waters could result in human health being affected if the mussels are contaminated with pollutants (Wright & Welbourn, 2002).

Toxicants and their uptake in the marine ecosystem

Marine pollution is an anthropogenic factor that may prevent the ecological sustainability of the marine environment within the carrying capacity of marine ecosystems (Schobben & Scholten, 1993). The type of pollutant (toxicants such as petroleum, pharmaceuticals, pesticides and metals) depends on its nature and source (Connell *et al.,* 1999; Fent, 2004). According to Connell *et al.* (1999), toxicants are substances that are considered to be toxic in relatively small doses and do not originate from animals and plants and as such are not natural agents. These authors have noted that there are substances that are discharged into the environment and therefore have a specific relationship in the natural environment. These substances are generally discharged from various types of industries in the forms of petroleum hydrocarbons, metals, acids, alkalis, solvents, amongst others. As such, toxicants comprise a wide range of chemicals and their derivatives (Fent, 2004). It should however be noted that not all these substances are considered to be ecotoxicants as some dosages are not detrimental to the health of organisms and ecosystems. What is of importance is the bioavailability of chemicals as this is considered the critical parameter for uptake and accumulation at target sites in organisms (Fent, 2004).

The main sources of ecotoxicants are from man-made sources. According to Connell *et al.* (1999), sewage is a common source of low dosage ecotoxicants. These sources, together with storm water systems, provide low-dosage but consistent sources of ecotoxicants such as metals. Given the growing demand for low cost products in industries, synthetic compounds are becoming more widespread and their effects greater (Eisler, 1981; Den Besten, 1998; Connell *et al.*, 1999; Ellerby & Bredesen, 2000; Wright & Welbourn, 2002). Metals however, occur naturally in the environment but anthropogenic sources (*e.g.* fossil fuel and waste burning, mining and ore processing, chemical production) are responsible for most concentrations observed in coastal environments (Besada, *et al.*, 2011). Furthermore, natural processes, such as run-off from mineralised areas and upwelling of oceanic water can also be the cause of high concentrations of metals in coastal organisms (Besada, *et al.*, 2011).

Irrespective of its source, the nature of ecotoxicants is that they have the potential to negatively influence organisms and ultimately ecosystems. The initial effect after

discharge of toxicants is important as it determines the nature of dispersal in the environment. It is from the environment that ecotoxicants are taken up by organisms. However, understanding the specifics of how this happens is difficult. In ecotoxicological research, cellular effect studies (such as mechanisms of toxic actions) are important because the main interaction between chemicals and the environment happens at the surface of or in cells (Fent, 2004). Bioavailability and uptake is determined by the chemical/biochemical characteristics of the toxicant and phase it is in. The toxicity of toxicants depends on a number of factors including its absorption, distribution, metabolism and excretion (Kakkar & Jaffery, 2005) and adaptive responses (Fent, 2004). Dispersal of toxicants can take place via air, water or soil. Furthermore, if a compound is water soluble, it tends to favour aquatic phases such as the sea (Connell *et al.*, 1999).

To gain access to an organism, toxic materials need to pass through at least one layer of cells. In simple unicellular organisms much has been investigated about the kinetics of chemical bioaccumulation (Wright & Welbourn, 2002). The movement of toxicants however becomes more complicated in higher order organisms with complicated membrane structure and function. In marine invertebrates, the uptake is mainly across the digestive epithelial layer that tends to be iso-osmotic to the surrounding medium (Fent, 2004). This creates an environment conducive to toxicants transversing the epithelial layer (Kakkar & Jaffery, 2005), in particular the digestive system (Fent, 2004; Kakkar & Jaffery, 2005). Further movement of toxicants in marine invertebrates is in the gills where gaseous exchange takes place. This is because the epithelial layers in the gills are very thin to facilitate gaseous exchange. In addition to this, the gills have a large surface area to facilitate gaseous exchange and food absorption in filter feeders efficiently. These conditions make for an ideal situation for easy movement of toxicants from the environment into the marine invertebrate.

1.3. Methods used to assess the status of marine environments

There are numerous methods that are used to assess the status of marine environments (Diamant & Westernhagen, 1999). Historically, assessments of the

health of the environment was based on pure chemistry-based approaches. Recent research (past 20 years) has however moved towards ecosystem health centred research known as the discipline of ecotoxicology. Since its first use in the late 1970's, the term ecotoxicology has been defined as the study of the effects of anthropogenic toxicants on ecological systems (Preston, 2002). Ecotoxicology deals with the interactions between environmental chemicals and biota, and focuses on adverse effects at different levels of biological organisation (Preston, 2002; Fent, 2004). It originated from different disciplines (toxicology, applied ecology and environmental chemistry) and is considered an interdisciplinary science (Fent, 2004). This approach was deemed necessary considering the complexity of anthropogenic effects on marine ecosystems.

To illustrate this point, decreasing water and sediment quality can result in a reduction of productivity of marine organisms. However, chemical analysis of pollutants in ecosystems alone cannot provide evidence for toxicological consequences in biota (Lam & Gray, 2001; Fent, 2004). This is because causal relationships between effects of contaminants and changes in populations or communities are often difficult to establish (Den Besten *et al.,* 2001). It is therefore important to develop methods for the identification, estimation, comparative assessment and management of risks posed by chemical pollutants to the environment. Hence the measurement of biological (and ecological) effects of marine pollutants has become vital for the assessment of the quality of the environment (Cajaraville *et al.*, 2000; Lam & Gray, 2001).

According to Lam and Gray (2001), measuring chemical and toxic pollutant concentrations alone will provide inadequate information and it could be considered preferable to measure risks and benefits associated with specific environmental circumstances or contamination/pollution scenarios. Measuring risks and benefits associated with pollutants can be done by conducting Ecological Risk Assessments (ERA) where organisms are exposed to potentially toxic chemicals which in turn will provide information on the risk of that exposure. The resulting information will be the ratio between a Measured Environmental Concentration (MEC) and the Predicted Environment Concentration (PEC) or Predicted No Effect Concentrations (PNEC). According to Lam and Gray (2001) if the risk quotient is greater than one

(environmental concentration is greater than level that will elicit an effect), there is a strong likelihood that there would be an impact.

A vital component of ERA is the ability to determine the critical stress levels for a biological system below which no adverse effect could be expected (Lam & Gray, 2001). Calculations of PNEC's can be based on ecotoxicological investigations where test organisms are exposed to defined levels of toxic stressors and their observed response observed and quantified under controlled conditions (Preston, 2002). Internationally, such tests are an integral part of ERA's and used to guide local environmental legislation.

In this respect, biomarkers are considered useful tools since they provide a means to detect exposure to contaminants and can be used as diagnostic tools to interpret observed effects at the organism level. Bioindicators are used as diagnostic tools at population level (Den Besten, 1998; Fent, 2004; Kakkar & Jaffery, 2005).

1.4. Biomarkers

Biomarkers are typically defined as quantitative measures of changes in the biological system that respond to either (or both) exposure to and or doses of xenobiotic substances that lead to biological effects (Schlenk, 1999; Lam & Gray, 2003). The term biomarker is generally confined to suborganism changes in organisms and provide early warning signals for environmental deterioration (Lam & Gray, 2003).

According to Kakkar and Jaffery (2005), biomarkers that have been validated in laboratory studies provide direct measures of actual effects of chemicals on living organisms in the field. Stress as a result of exposure to a pollutant usually results in biological responses that can serve as biomarkers. Biomarkers should therefore indicate that organisms have been exposed to pollutants (exposure biomarkers) and/or the magnitude of the organisms' response to the pollutant (effect biomarkers / biomarkers of stress) (Cajaraville *et al.*, 2000). Biomarkers of exposure are valuable to understand environmental carcinogens because ways to identify risk factors for disease outcomes with greater sensitivity are possible (Kakkar & Jaffery, 2005).

According to these authors, intervention to reduce long-term risk (control of exposure to pollutant) requires early detection of signs of toxicity and to attain this, the correct biomarker need to be used.

According to Van der Oost *et al.* (2005), when the pollutant dose or exposure time exceeds a certain threshold, the pollutant-responsive biomarker signals change from the normal range (in an unstressed situation) and leads to the expression of a multiple-effect situation at higher levels of biological organisation. Therefore, an important feature of cellular biomarkers is that they have the potential to predict changes at higher levels of organization (Cajaraville *et al.,* 2000; Van der Oost *et al.*, 2005).

Most biomarkers are measurements of body fluids, cells or tissues that indicate biochemical or cellular changes. As a result biomarkers are considered “early warning signals” that can be used to predict the health status of the environment. Furthermore, once validated in the laboratory, biomarkers can provide direct measures of actual effects of chemicals on living organisms (Kakkar & Jaffery, 2005). According to Van der Oost *et al.* (2005), the most promising feature of biomarker investigation is the early indication of potentially toxic effects. Therefore, by screening biomarker responses, information can be gathered about organism toxicant exposure and stress. According to Kakkar and Jaffery (2005) manifestation of toxicity of metals is a function of: specific metal; form of metal; level of exposure and period of exposure.

Biochemical responses are measurements of subcellular biochemical changes or molecular response for specific chemical or physical perturbations (Wright & Welbourn, 2002). Molecular markers commonly used in monitoring programmes include acetylecholinesterase inhibition, stress proteins, cytochrome P450 system induction, metallothionein induction, lysosomal membrane destabilisation and peroxisome proliferation (Cajaraville *et al.,* 2000). These biomarkers can be used to evaluate exposure to and effect of different contaminants (metals, organic xenobiotics and organometallic compounds) and they can be measured using different approaches (biochemistry, cytochemistry and immunochemical methods) (Cajaraville *et al.,* 2000; Wright & Welbourn, 2002).

According to Cajaraville *et al.* (2000), enzyme activities can be used as stress indicators or general biomarkers of stress. Deleterious effects of pollutants such as metals may result from direct toxic action on tissue or due to subtle changes in homeostatic mechanisms, such as the immune system (Auffret & Oubella, 1997). The immune defence of mussels comprises cell-mediated mechanisms, in which haemocytes or blood cells play a vital role (Cheng, 1981). The haemocytes are involved detoxification of metallic and organic pollutants via the endolysosomal system (Cajaraville & Pal, 1985). According to Kerher (1993) and Gómez-Mendikute *et al.* (2002), pollutants are capable of forming reactive oxygen species (ROS), resulting from univalent reduction of oxygen, that can damage most cellular components (Regoli *et al.*, 2002). In normal conditions, cells contain a complex network of antioxidant defence that scavenges ROS and avoids damage related to their reactivity (Halliwell & Gutteridge, 1984; Cajaraville *et al.,* 2000). However, in some instances of ROS production the protection offered by antioxidant defence mechanisms may be exhausted and lead to oxidative damage to tissue macromolecules such as DNA, proteins and lipids (Cajaraville & Pal, 1985).

Antioxidant defence includes both enzymatic and non-enzymatic antioxidants (Box *et al.*, 2007). The antioxidant system involves enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSX-PX) that act by detoxifying the generated ROS. Glutathione reductase (GR) catalyses the reduction of glutathione disulfide (GSSG) to glutathione (GSH) contributing to the maintenance of the cellular redox status (Box *et al.*, 2007). Organisms have adapted mechanisms to guard against the harmful effects of toxicants by decreasing ROS production by activating the antioxidant system. However, all ROS are harmful to organisms at elevated concentrations. The levels or activities of antioxidants therefore are potential biomarkers as they express contaminant-mediated effect on the organism (Box *et al.*, 2007).

1.5. The ecological role of mussels and their use as biomonitors of pollution stress

Biomonitors refer to organisms that can indicate the effects of exposure to toxicants (Van Gestel & Van Brummelen, 1996; Schlenk, 1999). Wright and Welbourn (2002) refer to the term biological indicators and biological monitors which are defined based on the types of biological responses in terms of their application to toxicology. These are considered to be (a) the attainment of a physiological, biochemical, metabolic or morphological end-point and (b) the accumulation in tissue of a chemical substance.

According to Hammond and Griffiths (2006), five mussel species are common along the southern African coastline. The brown mussel *Perna perna* is the dominant species on the sub-tropical east and warm-temperate south coasts, is absent along the west coast of South Africa, then reappears in Namibia. The indigenous black mussel *Choromytilus meridionalis* and ribbed mussel *Aulacomya ater,* as well as the introduced Mediterranean mussel *Mytilus galloprovincialis,* are all abundant in the cooler waters of the South African west coast, but extend along the south coast to varying extents (Figure 1.1).

Although it is an alien species to the region, *M. galloprovincialis* plays an important role in the ecology of intertidal communities in South African rocky shores. This alien species is thought to have been introduced to the southern African coast in the late 1980's and now the dominant intertidal species on the Cape west coast from Cape Point to southern Namibia (Griffiths *et al.*, 1992).

Mussels are important components of diets of fish, rock lobsters, starfish, predatory whelks and octopus (Griffiths & Branch, 1997). The increasing dominance of *M. galloprovincialis* has also resulted in rapid removal of particulates from the water, which may deprive other filter-feeders of nutrients. *M. galloprovincialis* have much higher rates of filtration than other mussels and this could result in the filtered material being made available to benthic detritivores (in the form of mussel faeces) (Griffiths *et al.*, 1992).

Since mussels are sedentary filter feeding organisms, they may accumulate metals if exposed to high concentrations thereof (Torres *et al*., 2002; Box *et al.*, 2007). Because of the sedentary nature of mussels they have been used as sentinel

organisms in monitoring programmes (*e.g.* “Mussel Watch” programmes) as they have the ability to tolerate exposure to an array of pollutants (Domouhtsdou & Dimitriadis, 2001). The mussels are considered ideal indicator organisms because they are ubiquitous, sedentary filter feeders inhabiting coastal and estuarine areas (Pipe *et al.*, 1999). Mussels are therefore prone to bioaccummulate contaminants in their tissue (Box *et al.*, 2007; Jena *et al.*, 2009). The accumulated contaminants have a wide array of adverse biochemical and physiological impacts on mussels (Jena *et al.*, 2009). The effects in the mussels include reduced filtration rates, altered gill structures, imbalance of Ca^{2+} signalling pathway and oxidative damage (Torres *et al.*, 2002). Furthermore, since oxidative stress responses are directly related to cellular function, they may provide clear indications of local pollution status (Jena *et al.*, 2009).

1.6. Marine pollution investigations in South Africa

Although South Africa’s coastal environment is considered to be pristine (Branch *et al.,* 2010), the effects of pollutants in the region are not known (O’Donoghue & Marshall 2003; O’Donoghue & Marshall, 2006). This is the case despite South African coastal regions undergoing urban and industrial development that has resulted in localised release of effluent. According to O’Donoghue and Marshall (2003), there has been an apparent neglect of scientific investigation of marine pollution in recent times. This is surprising considering South Africa’s strong culture of marine research. The Department of Environmental Affairs (DEA) and the Department of Agriculture, Forestry and Fisheries (DAFF) are the government agencies responsible for the management of marine research, not only nationally, but also regionally. Marine pollution research was intensively conducted up to the 1980’s (O’Donoghue & Marshall, 2003). However, given the restructuring processes in 2009, many key scientists in marine pollution are no longer employed by DEA and DAFF and there is a lack of even basic pollution studies in the region.

A comprehensive overview of marine pollution research output has been published by O’Donoghue and Marshall (2003). These authors noted near-normal curve of the number of publications, where publications rose sharply from the 1960’s to peak in

the early 1980's, and then fell equally sharply to a present day low level (Figure 1 of O'Donoghue & Marshall, 2003). These authors further noted that the types of pollution investigations were: ecotoxicology (40%), biomonitoring (42%) and bioindication (18%).

A literature review of biomarker investigations on marine organisms in South Africa has provided very limited information of such study types. O'Donoghue and Marshall (2003) noted that only one paper has been published on biomarkers by 2003. Marshall and Rajkumar (2003) investigated imposex development in two harbours in South Africa (Durban, Richards Bay) and the Knysna Lagoon. The results indicated that imposex was prevalent in South African harbour populations of *Nassarius kraussianus*, and thereby confirmed the bioavailability of tributyltin (TBT) in the areas investigated. Mills (2005) assessed the health of Richards Bay Harbour by studying spatial and temporal variations of bioaccumulation and biological responses in *Perna perna* using an active biomonitioring approach. Metal bioaccumulation was determined and responses measured using various biomarkers (acetyl cholinesterase inhibition, DNA damage, metallothioneins, cellular energy allocation, condition index and hydration index. The results indicated cellular energy allocation and condition indices were ideal biomarkers to detect and evaluate biological changes.

According to O'Donoghue and Marshall (2003), most investigations on marine pollution were done in the Western Cape, followed by the Eastern Cape, and then Kwazulu Natal (47%, 28% and 25%, respectively). The discrepancy in output was explained by an active marine research community in the Western Cape (University of the Western Cape, University of Cape Town and the Directorate: Marine and Coastal Management, now Oceans and Coast). Comparisons among pollutant types showed that most of the investigations concentrated on trace metals (35%), nutrient enrichment (23%) and polycyclic aromatic hydrocarbons (PAH) (16%).

The City of Cape Town is one of the largest urban centres of South Africa. The city is one of the three largest industrialized areas in South Africa and has characteristics of both the developed and developing world (van Beers & Graedel, 2003). The city and its surrounding areas are renowned for its high species richness, high beta and gamma diversity and high endemnity (Helme & Trinder-Smith, 2006). Considering the

increase in traffic of marine vessels around the Cape Peninsula, it is surprising that biomonitoring is poor (and hence is not keeping up with international trends). There has been a shift to the more frequent use of biomonitors to indicate levels of pollutants in the environment. Biomonitors in turn are used as models of biomarkers to consider cytological, physiological and morphological effects on attributes of organisms (O'Donoghue & Marshall, 2003). A biomonitoring investigation therefore not only provides insight (albeit limited) into levels of pollutants in an area, but also considers the health of organisms (at cellular and tissue level).

1.7. Rationale and objectives of the study

Decreasing water and sediment quality can result in a reduction of productivity of marine organisms. Cape Town is a densely populated coastal metropolis with a high growth rate. With this growth rate comes more sources of pollution that can cause excessive contamination of the coastline.

This study assessed the levels of metals in both the intertidal zone (water and sediment) and the tissue of *Mytilus galloprovincialis* along the west coast of the Cape Peninsula, Cape Town. The thesis investigates the effects of metals at the cellular level with the intention to develop a biomonitoring system that could form part of marine biomarker research in Southern Africa.

The following research questions were addressed:

- What is the extent of metal pollution in intertidal waters and sediment of the west coast of the Cape Peninsula?
- What are the levels of metal bioaccumulation in mussels along the west coast of the Cape Peninsula?
- Can antioxidant response in mussels exposed to metals in the field and in a controlled laboratory setting be used as a biomarker of toxic stress?

The specific objectives of the study were:

- To determine the current levels of metals in the waters and sediment off the west coast of the Cape Peninsula and how do these compare to previous years.
- To ascertain the historical levels of metal bioaccumulation in *Mytilus galloprovincialis* collected as part of the Mussel Watch Programme (1985 – 2005).
- To determine the current levels of metal bioaccumulation in *M. galloprovincialis* along the west coast of the Cape Peninsula.
- To determine whether antioxidant responses in a controlled laboratory experiment can be used as biomarkers of metal exposure in *M. galloprovincialis*.
- To determine if antioxidant biomarkers can be applied as tools to assess the effects of metal-induced stress in *M. galloprovincialis* off the west coast of the Cape Peninsula.

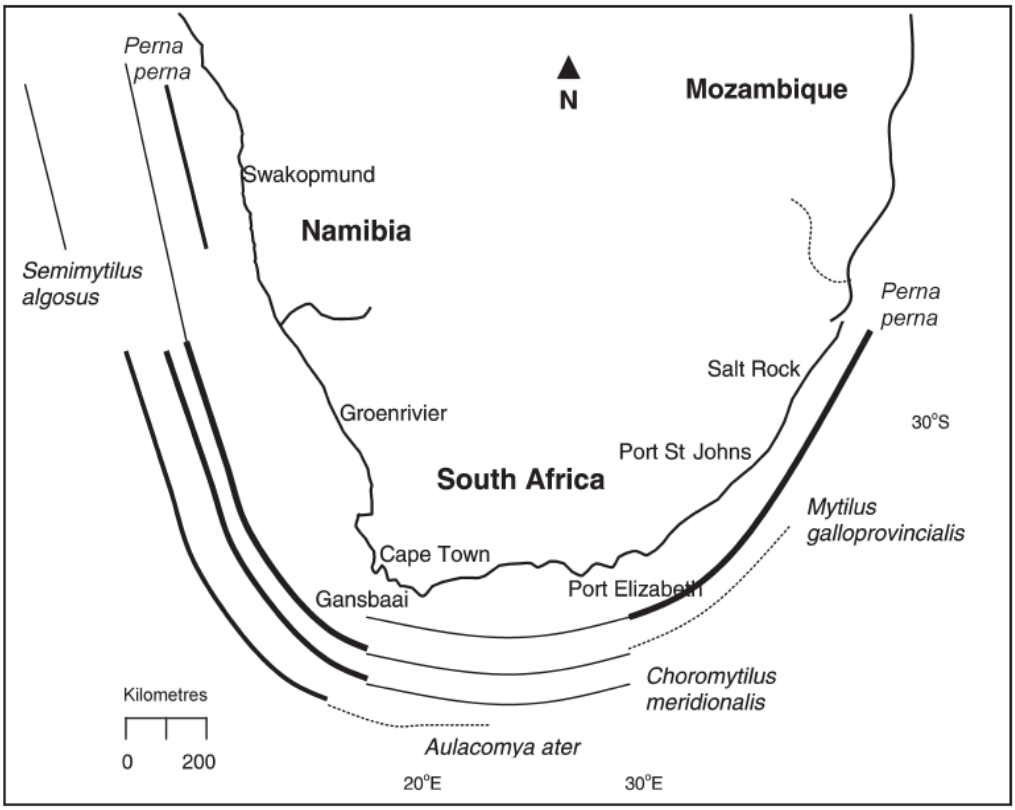

Figure 1-1. Map of southern Africa showing the location of the distribution of the dominant mussel species. The thickness of lines indicates relative abundance. (Source: Hammond & Griffiths, 2006).

Chapter 2

AN ANALYSIS OF HISTORICAL MUSSEL WATCH PROGRAMME DATA FROM THE WEST COAST OF THE CAPE PENINSULA, CAPE TOWN

2.1. Introduction

Dramatic degradation of the marine environment has led to changes in ecosystem structure and function. This is particularly evident in coastal areas where often these changes are evident in the change in intertidal structure. Such changes have even been observed by coastal (subsistence) communities, especially when resources they have been fished/removed for very long periods prior, have disappeared. Such trends were evident globally, and scientists started asking questions about the impact of pollutants on coastal systems. In the 1970's the need to investigate the levels of pollutants became evident (Viarengo & Canesi, 1991). In an attempt to determine the nature and extent of marine coastal pollution, a Mussel Watch Programme was proposed by Goldberg (1975). The Mussel Watch Programme (MWP) was initiated because the effects of marine pollutants were recognized but was considered difficult to determine (Goldberg, 1986).

According to Farrington (1983) bivalves are considered ideal to be used as a surveillance tools to monitor coastal pollution because they have a widespread distribution across the world's coastal waters; are sedentary and thus effective in providing the pollution status for a given area; concentrate many pollutants by factors of a thousand to a hundred thousand; because the substances of concern are measured in the bivalve, a measure of their biological availability is obtained; they often occur in large populations such that sampling on an annual basis does not threaten their survival; they appear to be especially resistant to pollutants and often survive in areas where other organisms are eliminated; they can readily be transplanted from one area to another; they are commercial products (oysters and mussels) and are consumed extensively in some areas of the world, and hence pose human risk.

Goldberg (1986) also reported that mussels (bivalves) were ideal for monitoring of metal pollution because they are easier to handle, store and ship; the problem of the included solid phase in the gut regions is eliminated and the biological half lives of metals appear to be of the order of years to parts of years.

The Mussel Watch Programme (MWP) started in South Africa in 1985. Prior to this, similar small scale projects were carried out in South Africa to monitor metals in mussels (Orren *et al.* 1980) but this was done in isolation to that done in other parts of the world. The intention for the development of the MWP in South Africa was therefore to develop a means of monitoring the health of the coast and to be able to compare the data obtained with international trends. The samples have been collected since 1985, but unfortunately publications in accredited sources are lacking. Hence the value and effectiveness of the MWP in South Africa is relatively unknown.

2.2. Description of the study area and study sites

The Cape Peninsula is largely rocky, mountainous and dominated by the Table Mountain chain (van Herwerden & Bally, 1989). According to these authors, the Cape Peninsula has been separated from the high ground to the north and east by a band of low-lying sandy ground known as the Cape Flats. A series of mountain ranges extends from Table Mountain (1086 m), past the Constantiaberg (928 m) and Muizenberg (507 m) ranges to the Swartberg (678 m), south of Simons Town. Historically, urban development has centered on the slopes of Table Mountain, initially starting around the safe anchorage of Table Bay, and then gradually spreading southwards, mainly along the eastern sides of the Table Mountain chain. According to van Herwerden and Bally (1989), the shoreline along the Cape Peninsula is dominated by rocky shores along the mountainous section of the Peninsula, interspersed with pocket beaches of sand or mixed sand and rock. The geology of the area consists of mainly the Malmesbury group with granite underlying most of the western part of the Peninsula (Glass, 1981).

The area falls within a Mediterranean-type climatic region, typified by winter rainfall from successional cold fronts from the west and dry southeasterly winds during the summer. Winter frontal systems cause north and westerly winds. The annual mean temperature in the region is 17°C (range ±10°C). Because it is in a winter rainfall region, the area receives the bulk of its mean annual precipitation of between 500-700mm mainly during the months of April to August (Shannon, 1985).

A description of the area between Bakoven and Blouberg (this includes sites 3, 4 and 5 of this study) has been described by Quick & Roberts (1992). The bed rock in Table Bay belongs to the Malmesbury group which is characterized by shales, slates and minor sandstone). These rocks are exposed intermittently at Bloubergstrand and again between Moullie Point and Sea Point. South of Sea Point to Camps Bay is an intrusion of granite (Woodbourne, 1983). The sea bed of Table Bay comprises partially exposed bedrock which is covered by sediments which range in size from fine to course sand. The area between Bloubergstrand and the harbour comprises mainly fine sand that is further characterized by a narrow band of very fine sand between the harbour and Rietvlei (site 4). The source of this fine sand is likely to be the weathering of the Malmesbury group rocks (Woodbourne, 1983) as well as seasonal output from stormwater outfalls (Quick & Roberts 1992).

Bathymetry indicates that the seafloor in Table Bay slopes gently from east to west (Quick & Roberts, 1992) and has a small ridge to the west of Table Bay (Woodbourne, 1983). South of Moullie Point (sites 1 and 2) is more steeply sloped than the shoreline of Table Bay resulting in a narrow continental shelf. This has implications for upwelling in the area and hence potential sources of metals to the area. According to van Herwerden & Bally, (1989), the area between Noordhoek and Cape Point (site 1) is generally rocky with numerous small indentations and sandy stretches. The area is first backed by high mountain ranges and then by a series of smooth rounded hills and finally the steeper peaks at Cape Point. Within the 50 m bathymetric contour (which may extend as far as 7.2 km offshore, the bottom is generally uneven with a series of rocky shoals (SAN HO, 1979).

Currents at sites 3 to 5 are generally wind driven and there are negligible influences of outside shelf currents, such as the northward flowing Benguela Current (Shannon, 1985) and inner shelf currents (Neshyba *et al.* 1989). Despite the negligent influence of currents, the erection of the docks in Table Bay has resulted in a major effect on sediment transport and coastal erosion along the Table Bay shoreline (Quick & Roberts, 1992). The reflection of wave energy from the vertical harbor structure has resulted in erosion of the shoreline to the west and north. The rate of erosion has decreased since the 1980's (Rosenthal, 1991). The construction of the harbour however has had two further impacts on sediment dynamics in Table Bay (Quick & Roberts, 1992). Firstly, the construction has caused the northward movement of sand to be deflected offshore and secondly, a large volume of the offshore and nearshore sand reservoir was removed by dredgers during the construction.

The residence time of sediments within Table Bay is approximately four days. According to Quick and Roberts (1992), this is long and indicates that flushing within Table Bay is poor. This was noted as vital when taking into consideration the assimilation of anthropogenic wastes within the area. According to Quick and Roberts (1992), the annual surf zone temperature along the west coast of the Cape Peninsula ranges from 9-21 °C. During winter, the temperatures range between 15-16 °C and rarely vary more than a degree either side of this norm. During summer however, the average surf zone temperature falls to 13°C, but these temperatures in summer could fluctuate over the entire range of temperatures within a space of a few days due to upwelling that is driven by the southeasterly winds (Jury, 1985). The closest pronounced upwelling centers along the Cape Peninsula are Cape Columbine to the north and Oudekraal to the south (Taunton-Clark, 1982). Once upwelling has occurred at Oudekraal (between sites 2 and 3), the cold upwelled water may be advected northwards, and transported to Table Bay. An understanding of the effects of temperature and the presence of nutrient-rich upwelled water has implications for understanding the dynamics of nutrients in the area.

The main objective of this study was to analyze the MWP data (1985-2008) to ascertain if there were any temporal and spatial changes to metal concentration in the mussels along the western coastline of the Cape Peninsula. The sites were

classified according to anthropogenic influences, as either conserved coast (Olifantsbos, also considered to be the reference site) or contaminated coasts (Hout Bay, Green Point, Milnerton and Bloubergstrand). Table 2-1 provides an overview of the selected sites.

2.3. Materials and Methods

This study analysed the historical data from the Mussel Watch Programme (MWP) in South Africa from 1985 to 2008. Samples were collected in spring and autumn during spring low tide as part of routine monitoring that took place along most of the coastline of South Africa (from Saldanha Bay to Richards Bay). *M. galloprovincialis* were collected and analysed for metals (µg/g dry weight) by the Department of Environmental Affairs. Although sample data were extracted from numerous MWP sites in the Cape Peninsula, only samples collected from 5 sites along the west coastline the Cape Peninsula are presented here (see Table 2-1). Seven metals were analysed (Cd, Cu, Pb, Zn, Hg, Fe and Zn).

2.3.1. Mussel sampling and metal analysis

Prior to 1995, all Mussel Watch Programme (MWP) samples (n=702, average mussel length=60.8 mm) were collected and processed according to the methods used by Watling & Watling (1976). Soft tissue of *Mytilus galloprovincialis* were weighed and then dried at 105°C for 48 h. The dried, weighed tissue was digested with redistilled, Analar-grade nitric acid and the solution was evaporated to dryness. The residue was redissolved in a 4:1 nitric-perchloric acid mixture and the solution dried at about 250°C. This residue was then dissolved in 10 mL of 0.1 mol/L Nitric acid. Metal concentrations in solution were determined by atomic absorption spectrometry. The results were expressed as metal content (µg metal/individual) and as metal concentration (µg/g dry tissue).

After 1995, mussel samples (n=802, average mussel length=62.2 mm) were detached from rocks and placed in plastic bags. On return to the laboratory, they were depurated in tanks filled with flowing sea water for 24 hours, whereafter they

were frozen. Samples were weighed and then de-shelled with all their contents emptied into marked containers and re-weighed. All samples were then refrigerated once more and when thoroughly frozen, were placed into a freeze-dryer for approximately 3 days (or until mussel tissue was flaky and dry). The anhydrous product was then placed into a pulverizer to obtain a homogenous sample.

Once pulverized, the sample was digested using the microwave method. The samples were prepared for digestion by placing 0.5 g samples into a quartz insert. To this, 6 mL concentrated nitric acid and 1 mL H_2O_2 (hydrogen peroxide) was added and allowed to stand for a few hours. The quartz incerts were then placed into the segmented rotor block of the microwave and digested. After digestion, the vessels were allowed to cool before opening.

Stannous Chloride ($SnCl_2$) solutions (25% w/v) were prepared by finely crushing 62.5 g tin chloride granules and adding it to 187.56 mL 20% hydrochloric acid in a 250 mL volumetric flask. This solution was then heated on a hot plate until all the powder was dissolved and the solution clear. The blank was prepared by digesting 6 mL nitric acid and 1 mL perchloric acid. The purpose of the blank was to measure the contribution made by the reagents during the analysis. Reference material was used to ascertain whether or not the Atomic Absorption Spectrophotometer and digestion methods gave reliable readings. The blank was prepared by digesting 0.5 g Dorm-2 reference material, 6 mL nitric acid and 1 mL perchloric acid in the microwave.

2.3.2. Statistical analysis

All statistical data analysis was done using Statistica 10^{TM}. The effects of time (annually and per season) and location on metal concentration in mussels were analysed, using one-way ANOVA for analysis of single factors (year, season or site) and multiway ANOVA to test the effects of time (year and season) and location (distance from control site) on metals (Cd, Cu, Zn, Pb, Hg, Fe and Zn). Prior to the use of the parametric tests, the data were tested for normality and homogeneity of variances using Kolmogorov-Smirnov and Levene's tests respectively (Townend, 2002). If the data did not meet the assumptions of the tests, the data were log_{10}-transformed prior to analysis. For ANOVA analysis, post-hoc Tukey tests were done.

Error bars in graphs indicate standard error of the mean. Differences between seasonal metal concentrations were done using one way ANOVA and significant differences indicated at $p<0.05$.

From 1990, only one sample was collected per site. To enable ANOVA analysis to be statistically valid, MWP sampling stations were combined to representative sites (Table 2-1), but these were applied to all data.

2.4. Results

2.4.1. Metal concentrations in mussels

Metal concentrations showed highly variable values when all data per metal were combined (Table 2-2). Metal values ranged from not detected (ND) to 1625.56 µg/g dry weight Zn (Table 2-2). The highest mean values recorded were for Zn 186.16 ± 125.61 followed by Fe 129.32 ± 163.38 µg/g dry weight. The high variability (indicated by the SD values) in Table 2-2 validated the need to normalise the data and hence supports the $\log_{10}$ transformation that was applied to the data.

The remainder of the mean metal concentrations were below 7 µg/g dry weight per metal. There was a highly significant difference between all metals for the entire period of the study 1985 to 2008 ($p<0.001$). The decreasing order of metals for all the sites combined was Zn>Fe>Cd>Cu>Pb>Mn>Hg.

The mean concentrations of metals in soft tissue of *M. galloprovincialis* for the period 1985 to 2008 at all sites are shown per year (Figure 2-1) and per season (autumn and spring 2010) (Figure 2-2).

Copper concentrations were low but variable, with one peak in 2000 when the mean Cu recorded was 23.24 ± 20.88 µg/g dry weight (Figure 2-1a). The values ranged from nd (2004-2005) to 101 µg/g with a mean of 4.39 ± 4.96 µg/g (Table 2-2). The concentrations for all the stations were generally below 10 µg/g dry weight. There was a highly significant difference in Cu concentration between years ($p<0.001$, $n=1318$). The concentrations of autumn and spring 2010 samples differed significantly ($p<0.05$) (Figure 2-2a).

Cadmium levels ranged between nd and 39.1 µg/g dry weight. Mean concentrations of Cd were low for most of the study period (6.17 ± 5.05 µg/g), except for 2000, where higher Cd concentrations were measured (Figure 2-1b). This was followed by a decline in Cd concentrations after 2000 to 2008 (Figure 2-1b). The ANOVA suggested that there were highly significant differences in Cd concentrations in years for the period 1985 to 2008 ($p<0.001$, $n=1309$). There were also generally significantly lower Cd concentrations ($p<0.001$) in spring than autumn (Fig 2-2b) within the study period.

Mercury measurements were only done from 1985 to 1995. The Hg readings ranged from nd to 0.89 µg/g dry weight, with the mean concentration being 0.16 ± 0.14 µg/g dry weight. Mercury concentrations were variable (Figure 2-1c) with a three-fold increase in 1987. There was a highly significant difference in Hg between years when Hg was sampled ($p<0.001$, $n=480$). Although readings were lower in autumn, there were no significant differences in Hg concentrations between autumn and spring (Figure 2-2c).

Iron was recorded in mussels from 1987 to 1988 and then again from 1996 to 2003. The highest Fe concentrations were recorded during the 1996-2003 period. There was an increase in Fe concentrations from 1996 until 2001 and thereafter Fe concentrations decreased. The concentration in 2000 was significantly higher than previous years ($p<0.001$). Fe concentrations in *M. galloprovincialis* ranged from nd to 1309 µg/g dry weight. Mean Fe values for the study period was 129.32 ± 163.38 µg/g dry weight. There was a highly significant difference between annual Fe measurements ($p<0.001$, $n=534$) (Figure 2-1d).

Lead measurements during the MWP were variable as values ranged from nd to 427.6 µg/g dry weights (Figure 2-1e). The mean Pb concentrations in mussels were 5.07 ± 16.47 µg/g dry weight. There was a highly significant interannular Pb variation ($p<0.001$, $n=1160$). Although Pb values were higher in autumn, the differences between autumn and spring were not significant (Figure 2-2e).

Manganese displayed similar concentration patterns as Fe. Values were low during the 1980's but peaked in 2000. Thereafter, Mn values decreased (Figure 2-1f). The Mn concentration in mussels ranged from ND to 64.7 µg/g dry weight. The average

Mn recorded in mussels from all stations was 4.20 ± 6.14 µg/g dry weight. The concentrations of Mn from 1996 was very low with only one spike (>20 µg/g dry weight) being recorded in 2000. There was a significant difference between annual Mn concentrations ($p<0.001$, n=544). Although lower Mn concentrations were recorded in autumn, there was no significant difference between autumn and spring concentrations (Figure 2-2f).

Zinc had relatively high concentrations from the start of the MWP (>200 µg/g dry weight) and then gradually decreased until 2003 (Figure 2-2g). After 2003, Zn concentrations decreased considerably compared to previous years. The mean Zn concentration for the study period was 186.16 ± 125.61 µg/g dry weight with the highest value being 1625.56 µg/g dry weight. Interannual Zn concentrations were highly variable and significantly different ($p<0.001$, n =1258) (Figure 2-1g). Spring Zn concentrations were significantly higher than autumn ($p>0.05$).

2.4.1.1. Site 1 (Olifantsbos and Noordhoek)

Copper concentrations were low but stable at site 1 (Figure 2-3, Table 2-2). Although samples of 1991, 1992 and 1999 were highly variable (Figure 2-3a), inter-annual variation was low. After 2000, there was a decrease in Cu recordings. The highest Cu value recorded was 27.17 with mean values of 6.56 ± 4.18 µg/g dry weight being recorded at site 1. There was highly significant ($p<0.001$) differences in Cu concentrations between years.

Cadmium concentrations at site 1 were generally stable with one peak in 2000 (Figure 2-3b, Table 2-2). The mean Cd concentrations were 6.56 ± 4.18 µg/g dry weight with 21.85 µg/g dry weight being the highest recorded for the site. An ANOVA analysis indicated that there was a highly significant variation in Cd concentrations per annum ($p<0.001$, n=107). Although Cd concentrations were higher in autumn (Figure 2-4b), the differences were not significant ($p>0.001$).

Mercury levels at site 1 were very low and mussel tissue samples were only collected until 1995 (Figure 2-3c). The Hg values ranged from nd to 0.350 µg/g dry weight with mean values of 0.12 ± 0.08 µg/g dry weight being recorded. There were highly

significant temporal variations in Hg concentrations ($p<0.001$, $n=24$) at site 1 (Figure 2-3c). Although higher Hg values were recorded in spring than autumn, the differences were not significant (Figure 2-4c).

Sampling for Fe at site 1 only took place from 1996 to 2007 (Figure 2-3d). The Fe concentrations ranged from ND to 976.5 µg/g dry weight with an average of 127.47 ± 181.10 µg/g dry weight (Table 2-2). One-way ANOVA indicated that annual concentrations differed highly significantly ($p<0.001$, $n=40$) but that there was no significant differences between seasons (Figure 2-4d). The Fe levels recorded were the second highest for site 1.

Lead concentrations were consistently low throughout the period 1985-2008 with only one peak in 2000 (Figure 2-3f). The highest Pb concentrations measured at site 1 was 76.35 and the average was 2.94 ± 10.61 µg/g dry weight. One-way ANOVA's showed that there was a highly significant difference between years ($p<0.001$, $n=88$) and no significant differences between seasons.

There was no Mn recorded prior to 1996 (Figure 2-3f). Thereafter Mn concentrations increased to 2000 and thereafter concentration decreased. The highest Mn recorded was 64.7 µg/g dry weight with an average of 4.26 ± 7.46 µg/g dry weight. ANOVA analysis showed that there was a highly significant annual difference between concentrations ($p<0.001$, $n=39$) but no significant difference between seasons.

Zinc concentrations were consistent throughout the period that samples were collected (Figure 2-3g). The highest Zn values recorded was 1625.6 µg/g dry weight, and the average recorded was 159.22 ± 161.97 µg/g dry weight. Zinc had the highest metal values recorded at site 1. The ANOVA showed that there was a highly significant annual difference between concentrations ($p<0.001$, $n=102$) but no significant difference between seasons (Figure 2-4g).

2.4.1.2. Site 2 (Six sampling stations within Hout Bay)

Metals sampled at site 2 (Hout Bay) recorded very low values for the period 1985 to 2008 for most of the metals sampled (Table 2-2, Fig 2-5). Only Fe and Zn recorded very high values during the study period (however, note the high maximum Pb value

recorded). Both Fe and Zn were low for most of the study period and Cd and Zn showed high inter-annual variability. The remainder of the metals showed consistently low values (Fig 2-5). Although low mean values were recorded for site 2, the maximum values were generally higher than those recorded at site 1. Metal concentrations from highest to lowest were as follows: Zn>Fe>Cu>Cd>Pb>Mn. The Zn values were consistently high for the study period (Fig 2-5g). Hg was not measured after 1995 and hence the results not included here.

One-way ANOVA analysis of the Hout Bay metal data indicated that there were highly significant ($p<0.001$, $n=761$) differences for all metals between years. One-way ANOVA showed that seasonal differences were significant for Cu, Cd, Hg, Mn and Zn ($p<0.05$) (Fig 2-6).

2.4.1.3. Site 3 (Sea Point, Green Point, Granger Bay)

Site 3 was situated along the western seaboard of the Cape Peninsula and included 4 sampling stations (Table 2-1). These areas are exposed to open ocean waves and are not large enclosed and protected bays (such as Hout and Table Bay). Very high maximum metal values were recorded for Zn (1306.550 µg/g dry weight) and Fe (1309.000 g/g dry weight) (Table 2-2). The order of mean values recorded was: Zn (221.906 µg/g) > Fe (161.966 µg/g) > Cd (6.928 µg/g) > Pb (7.296 µg/g) > Mn (4.280 µg/g) > Hg (0.208 µg/g).

The concentrations of Cu, Cd and Zn were consistent during the study period (Fig 2-7). The values of these metals decreased after 2000. Mercury was only recorded until 1995 and during the period when samples were collected, these were highly variable. Fe and Mn were only sampled after 1996. During the period 1997 to 2003 both Fe and Mn increased and then decreased from 2000 onwards. There was high variability in the Pb measured in mussels. One-way ANOVA of annual metal concentrations showed highly significant differences ($p<0.001$, $n=223$) between years. There were only significantly higher concentrations of Cd in autumn and significantly lower concentrations of Hg and Zn in spring ($p<0.05$, $n=223$).

2.4.1.4. Site 4 (various stations within Table Bay harbour)

Site 4 is situated within Table Bay Harbour, one of the busiest harbours in South Africa. The site includes four sampling stations within the harbour. Metal concentrations at site 4 were similar to that of site 3. Here too, Zn (668 µg/g) and Fe (1057.5 µg/g) had the highest maximum concentrations (Table 2-2). The order of mean metal concentrations from 1985 to 2008 were Zn (221.010 µg/g) > Fe (170.445 µg/g) > Cu (5.765 µg/g) > Pb (5.611 µg/g) > Cd (5.231 µg/g) > Mn (5.098 µg/g) > Hg 0.127 (µg/g).

Zinc was consistently high during the study period (Fig 2-9) and decreased dramatically after 2000. Mercury was only recorded until 1995 with highly variable records during this period. Fe and Mn were only sampled after 1996. During the period 1997 to 2003 both Fe and Mn increased and then decreased from 2000 onwards.

One-way ANOVA of annual metal concentrations showed highly significant differences ($p<0.001$, $n=223$) between years at site 4. There were only significantly lower concentrations of Cu and Zn as well as significantly higher concentrations of Cd in autumn ($p<0.05$, $n=223$) at site 4 (Fig 2-10).

2.4.1.5. Site 5 (Milnerton, Paarden Island and Bloubergstrand)

Site 5 was situated to the north of Table Bay and comprised three stations. The station at Milnerton was close to an outfall pipe that was adjacent to the Rietvlei wetland mouth, Paarden Island was at a river mouth adjacent to an industrial area and Bloubergstrand was close to an urban area that underwent rapid development of houses in the past few years. Site 5 metal concentrations were similar to those of the previous sites. The highest maximum metal concentrations recorded were Fe (1013.5 µg/g) and Zn (575.25 µg/g) (Table 2-3). The order of mean metal concentrations for site 5 was: Zn (159.303 µg/g) > Fe (133.044 µg/g) > Cd (7.541 µg/g) > Cu (5.880 µg/g) > Mn (5.779 µg/g) > Pb (4.424 µg/g) > Hg (0.090 µg/g).

There were consistent concentrations of Cu and Zn from 1985 to 2000 (Figure 2-11), whereafter the metal concentrations decreased. There were no records of Mn and

Fe analysis prior to 1995, and for Hg, there were no records after 1995. Highly variable concentrations of Hg and Pb were recorded for the study period.

One-way ANOVA analysis of annual metal concentrations showed highly significant differences ($p<0.001$, $n=181$) between years at site 5. There were significantly higher Cd concentrations in spring and significantly lower concentration of Zn in autumn ($p<0.05$, $n=181$) at site 5 (Figure 2-12).

2.5. Discussion

The effects of pollutants (including metals) on living organisms can be evaluated at different levels of organization (molecular, cellular, individual, population and community) (Viarengo & Canesi, 1991). Good interpretation of the data can be obtained by studying the effects of pollutants on individuals, with the aim of understanding and eventually predicting the possible consequences at higher levels (Bayne, 1986). The Mussel Watch Programme (MWP) was established to monitor the concentrations of pollutants (metals in the case of South Africa) with the intention to monitor and evaluate the potential adverse impact of these metals on the marine ecosystem in South Africa.

The results of this study indicated the levels of metals in mussels for the western coastline of the Cape Peninsula. The results were approximately the same for the individual 5 sites of the MWP (Table 2-3). For all data combined, the mean order of decreasing metal concentrations were: Zn > Fe > Cd* > Cu > Pb* > Mn > Hg* (*indicates non-essential metals). The order of concentrations was similar to that reported by Watling and Watling (1976) and it is in this order that the metals will be discussed.

Site 1 is the southernmost region of the Cape Peninsula where mussels are sampled for metal analysis for the MWP. Although currents are variable south of the Cape Peninsula, the current along the west coast is in a northerly direction (Shannon, 1985). Because this station is in the Table Mountain National Park (Cape Point Nature Reserve), the area should be considered free of pollution of anthropogenic origin as opposed to the remaining four sites that have some anthropogenic sources

of pollutants. As such it will also be referred to as a control site in later chapters and discussions within the thesis.

According to Eisler (1981), the highest concentration of Zn in the marine environment is found in filter-feeding molluscs. The relatively high Zn concentrations recorded in mussels during the MWP therefore supports this as the Zn concentrations were significantly higher than the other metals recorded ($p<0.001$, $n=1712$). The source of Zn may be from anthropogenic sources. It is unlikely for this to be the case at site 1 as this site is far from any sources of anthropogenic Zn. According to Moore (1981) however, Zn uptake is mainly from prey rather than from sea water. The high levels of Zn were therefore more likely to be from zoo- and phytoplankton sources as the continental shelf is very narrow in this area (Shannon, 1985). The mean levels of Zn detected at site 1 (134.2221 µg/g dry weight) were below the maximum limits allowed in foodstuff as set by the South African Bureau of Standards (SABS) of 300 µg/g (South Africa, 1994). What is of concern though is that for site 1, the maximum levels recorded exceeds the SABS maximum limit (1625 µg/g was recorded in 1999). Furthermore, there are no local comparative studies to illustrate whether the current Zn values are higher than normal. However, the Zn values recorded along the Cape Peninsula are similar to other MWP's and is slightly above the median World MWP value (130 µg/g dry weight) (Cantillo, 1998). According to Cantillo (1998), Zn concentrations above 200 µg/g dry weight are indicative of contamination. The higher Zn concentrations are thus indicative of an increase in Zn input into the environment. Zinc values higher than 200 µg/g accounted for 21% of the Zn values at site 1. However, the Zn values are higher than that of Henry *et al.* (1986), who recorded Zn values of 0.56 and 0.39 µg/g in mussels Granger Bay and Green Point, respectively (site 3), but they used different mussel species. The source of the zinc is uncertain and needs further investigation.

Iron had the second highest concentration reported for the study period and the mean concentrations of Fe for all sites reported in this study (129.315 µg/g) is lower than that reported in other investigations where mussels were also sampled (Shiber & Shatila, 1978; Kavun *et al.,* 2002). According to Giarratano *et al.* (2010), changes in marine Fe concentrations may be related to continental sources of Fe, as the major contributor to Fe is from rock weathering as a result of continental rainfall.

Potential anthropogenic sources of Fe are from fertilizers, industry wastes, atmospheric deposition, solid waste disposal units and run-off from urban areas (Pergram & Görgens, 2001). The Fe tissue values recorded in the present study suggest that there are no major anthropogenic sources of Fe other than from urban run off and the main source of Fe is postulated to be as a result of rock weathering due to higher rainfall in autumn (Figure 2-2d). According to Giarratano *et al.* (2010), Fe concentrations reported from their study came from natural sources as human activities were not responsible for Fe input into the system.

Cadmium concentrations (mean = 6.174 µg/g dry weight) were similar to that of Cu for the study period 1985 to 2008 along the west coast of the Cape Peninsula. However, the levels recorded in this study are higher than the recommended SABS of 3.0 µg/g (South Africa, 1994). The values are also higher than Cd values for mussels that were indicative of contamination (3.7 µg/g) set by Cantillo (1998). The levels for Cd were also higher than the 2.48 µg/g recorded by Henry *et al.* (1986) for Table Bay (sites 3 to 5). Cd values indicative of contamination suggested by Cantillo (1998) were found at all sites. Cadmium occurs at high levels in the environment due to anthropogenic sources (Chiffoleau *et al.,* 2001). Cadmium reactions cause various geochemical processes such as the solubilization of Cd on freshwater particles when these reach sea water. As a result, Cd becomes available to molluscs living close to fresh water sources (Chiffoleau *et al.,* 2001). This phenomenon could account for the higher levels of Cd at sites 2 to 5 as there are potential freshwater inputs such as river mouths and stormwater pipes, although a study on metal concentrations from Diep River (freshwater input into Milnerton) showed low Cd concentrations in both water and sediment (Shuping, 2008). This cannot however explain the high values in site 1. The postulated reason for high Cd values at site 1 could be due to site 1 being a combination of two stations, where the mean Cd concentration in Noordhoek was 7.653 µg/g and in Olifantsbos was 6.046 µg/g. Noordhoek is a coastal area that could have substantial input of freshwater due to high levels of urbanisation. An alternative explanation is that the sources of Cd were not from anthropogenic sources but from upwelled waters (Reyes, 1995). Whether the Cd was from anthropogenic or natural sources needs further investigation.

Of the sites sampled, Cd concentrations were the highest at sites 3 (6.928 µg/g) and 5 (7.541 µg/g). Both these sites are at the shoreward end of open coasts and could hence have been influenced by Cd from up-current and stormwater outflow pipe anthropogenic sources. According to Chiffoleau *et al.* (2001), Cd levels in organisms could be related to domestic and industrial effluents. Sites 3 and 5 are in close proximity to both domestic and industrial sources of effluents that could be a source of Cd at those sites.

Copper is an essential element in mussels as it forms part of blood proteins (Phillips, 1976). There was a significant difference in Cu concentrations between years as well between different sites. These results are in agreement with previous studies (Adler-Ivanbrook & Breslin, 1999). According to Cantillo (1998), the reproductive process requires high levels of Cu in the tissue to facilitate effective reproduction. Copper concentrations were low for all sites (Table 2-2) and there were no significant differences between seasonal Cu concentrations for all sites combined (Figure 2.2b). However, there were significantly higher Cu concentrations in mussels at site 1. This result could therefore suggest greater reproductive success at site 1. Given that site 1 is considered the reference site, there are less anthropogenic stress factors and could result in higher reproductive success rates.

The mean levels of Cu recorded for the entire study area (5.616 µg/g dry weight) were below the maximum limits allowed in foodstuff as set by the SABS of 50 µg/g (South Africa, 1994). According to Cantillo (1998), Cu concentrations above 10 µg/g dry weight in marine mussels are indicative of contamination. The results therefore suggest that the Cape Peninsula is not contaminated with Cu in the coastal environment. The tissue values are similar to that recorded by Mdzeke (2004) for False Bay where 5 µg/g dry weight at Kleinmond was recorded for the period winter 2000 to winter 2001. The data of the MWP is however higher than that recorded by Henry *et al.* (1986) for Table Bay (2.48 µg/g).

High levels of Pb are found in the tissue of shellfish that occur near sewer outfalls, heavy traffic, industrialized or densely populated urban areas (Pergram & Görgens, 2001). The mean Pb levels recorded along the west coast of the Cape Peninsula (5.065 µg/g) were above the maximum limits allowed in foodstuff as set by the SABS of 4.9 µg/g (South Africa, 1994). According to Cantillo (1998), Pb concentrations

above 3.2 µg/g dry weight are indicative of contamination. Values higher than 3.2 µg/g were recorded at sites 2 (4.628 µg/g), 3 (7.296 µg/g), 4 (5.611 µg/g) and 5 (4.424 µg/g). Site 2 represents Hout Bay and sites 3, 4 and 5 are in the northern part of the study area and represents Table Bay. Table Bay has a major port (and Hout Bay to a lesser extent) and is highly urbanised. The high level of Pb prior to 2000 could therefore be indicative of Pb in petrol and hence the runoff from vehicle emmissions. This postulation is supported by other studies of lead levels in the environment where Henry *et al.* (1986) recorded Pb levels of 28.8 and 14.3 µg/g in Granger Bay and the Black River mouth, respectively. The levels of Pb in mussels of the MWP decreased after 2000 (Figure 2-1e). According to Yan *et al.* (1997), mussels are not able to regulate the levels of Pb and as a result, Pb tends to accumulate in mussel tissue and may reach very high concentrations when ambient Pb concentrations are high. This provides evidence of using mussels as biomonitors of metal concentrations, given that they are able to accumulate the metals in their tissue.

Manganese is an element found in all animal tissue and is required as an enzyme cofactor or activator of a number of metabolic reactions (Cotzias, 1958). Although the metal is important in trace amounts, exposure to high concentrations could result in accumulation to toxic levels in tissue. As such, consumption of organisms with excessive Mn content could result in humans developing Parkinson's disease (Levy & Nassetta, 2003). More recently however, there has been growing concern about chronic, low-level exposure to Mn as a contributing factor to people developing Parkinson's disease (Levy & Nassetta, 2003). There are no tissue standards in South Africa for maximum concentrations for MWP data for Mn. The data collected for this study (4.199 µg/g) was however, much lower than other studies on Mn accumulation in mussels collected in Europe (Regoli & Orlando, 1994; Swann *et al.* 1998) and therefore it is concluded that Mn has probably not bioaccumulated in *M. galloprovincialis* in the Western Cape, to levels that would be toxic to these animals and to humans consuming them.

Mercury measurements in mussels were only done until 1995. The mean Hg levels recorded along the west coast of the Cape Peninsula (0.045 µg/g) was below the maximum limits allowed in foodstuff set by the SABS of 1.0 µg/g (South Africa, 1994).

Cantillo (1998) noted that Hg concentrations above 0.23 µg/g dry weight are indicative of contamination. However, none of the sites recorded Hg values higher than either of these guideline values.

Multivariate analysis (MANOVA) of the MWP data along the west coast of the Cape Peninsula revealed significant effects of year and site including the interaction between year and site (Table 2-8). for all the metals analysed except for the effect of site on Fe and Mn and suggests that both temporal and spatial effects can influence the level of metals in mussels. This needs to be taken into consideration when implementing a biomonitoring system and careful consideration needs to be taken in site selection and timing (periodicity and frequency) of data collection.

2.6. Conclusion

Metal concentrations in mussels have been measured in *M. galloprovincialis* since 1985 as part of the MWP. The monitoring programme is important as it provides some indication of bioavailable metals in the coastal environment. The results of this study focussed on metal concentrations in mussels along the western coastline of the Cape Peninsula and the results have indicated that the levels of metals have been highly variable within the mussels over the period. The results indicated that metal concentrations in *M. galloprovincialis* along the west coast of the Cape Peninsula did not increase over the study period and that for some metals, the level of metals decreased. The significant differences between years and seasons suggest that the MWP is providing useful information about the levels of metals in the tissue of the organisms. However, the sources of the high levels of contaminants need further investigation. Furthermore, other potential toxicants could also be affecting the organisms and it is proposed here that the MWP considers to broaden the scope of contaminants (*e.g.* PAH) as these might be having considerable impact on the health of the coastal marine ecosystem, in addition to the impact of metals.

Table 2-1. Site and station information of samples collected during the MWP. The stations were within 1 km of each other and were considered representative areas for metal concentrations in *M. galloprovincialis* of that area similar to that referred to in later chapters.

Name	Stations	Number of MWP stations
Site 1 (Control Site)	Olifantsbos, Noordhoek	2
Site 2	Hout Bay	5
Site 3	Sea Point, Three Anchor Bay, Granger Bay	3
Site 4	Table Bay	4
Site 5	Milnerton, Paarden Island, Bloubergstrand	3

Table 2-2. Mean metal concentration (µg/g dry weight), standard deviations (SD), maximum concentrations (µg/g dry weight) and number of observations (N) of Cd, Cu, Pb, Zn, Hg, Fe and Mn along the west coast of the Cape Peninsula for the study period 1985 to 2008 for all sites combined and individual sites sampled. * indicated data from 1985-1995 only.

	All sites		Site 1		Site 2		Site 3		Site 4		Site 5	
	Mean (±SD)	Max (N)	Mean (± SD)	Max (N)	Mean (± SD)	Max (N)	Mean (± SD)	Max (N)	Mean (± SD)	Max (N)	Mean (± SD)	Max (N)
Cd	6.174 (±5.0497)	39.100 (1309)	6.557 (±4.1757)	21.850 (107)	5.636 (±4.3671)	35.400 (525)	6.928 (±6.1040)	39.100 (216)	5.231 (±4.1139)	32.500 (170)	7.541 (±6.082)	26.520 (115)
Cu	5.616 (±4.1985)	43.920 (1318)	4.791 (±3.8450)	27.170 (107)	5.491 (±3.9110)	43.920 (535)	6.677 (±5.6930)	37.470 (215)	5.765 (±3.2376)	21.870 (171)	5.380 (±3.6108)	29.070 (114)
Pb	5.065 (±16.4741)	427.600 (1160)	2.940 (±10.6182)	76.350 (88)	4.628 (±21.6154)	427.600 (463)	7.296 (±13.514)	87.950 (204)	5.611 (±10.0004)	79.600 (160)	4.424 (±11.601)	85.350 (96)
Zn	186.164 (±125.6134)	1625.550 (1258)	159.223 (±161.9723)	1625.550 (102)	180.980 (±99.3602)	932.000 (525)	221.9064 (±159.074)	1306.550 (193)	221.010 (±119.2236)	668.000 (162)	159.303 (±82.7828)	575.250 (109)
Hg*	0.160 (±0.1415)	0.890 (480)	0.119 (±0.0882)	0.350 (24)	0.164 (±0.1534)	0.890 (248)	0.208 (±0.1660)	0.870 (91)	0.127 (±0.0770)	0.320 (54)	0.090 (±0.0434)	0.200 (22)
Fe	129.315 (±163.3829)	1309.000 (534)	127.470 (±181.1003)	976.500 (40)	102.715 (±93.3100)	873.000 (255)	161.9661 (±231.4680)	1309.000 (78)	170.445 (±165.0314)	1057.500 (57)	133.044 (±156.7584)	1013.500 (42)
Mn	4.1992 (±6.1449)	64.700 (544)	4.264 (±7.4673)	64.700 (39)	3.432 (±4.9474)	55.750 (268)	4.280 (±4.5756)	21.300 (77)	5.098 (±4.7080)	31.460 (56)	5.779 (±9.7950)	64.700 (42)

Table 2-3. Results of MANOVA based on mean metal concentration estimates of *M. galloprovincialis* per year and site. * denotes a significant effect at p <0.001, ** denotes p < 0.0001. # indicated data from 1985-1995 only.

Metal	Sources of variation	Sum of Squares	Degrees of Freedom	Mean Square	F Value	P Value
Cd	Site	5.3823	4	1.3456	21.587	<0.0001**
	Year	103.8174	23	4.5138	72.414	<0.0001**
	Site x Year	27.7945	92	0.3021	4.847	<0.0001**
Cu	Site	1.1204	4	0.2801	6.449	<0.0001**
	Year	91.5828	23	3.9819	91.681	<0.0001**
	Site x Year	29.2485	92	0.3179	7.320	<0.0001**
Pb	Site	6.9677	4	1.7419	28.248	<0.0001**
	Year	77.8149	23	3.3833	54.864	<0.0001**
	Site x Year	27.5208	92	0.2991	4.851	<0.0001**
Zn	Site	5.978	4	1.495	5.061	0.000472*
	Year	678.503	23	29.500	99.908	<0.0001**
	Site x Year	189.055	92	2.055	6.960	<0.0001**
Hg[#]	Site	0.016542	4	0.004136	7.0763	<0.0001**
	Year	0.436915	23	0.018996	32.5043	<0.0001**
	Site x Year	0.388591	92	0.004224	7.2273	<0.0001**
Fe	Site	3.1823	4	0.7956	2.0102	0.090676
	Year	531.9323	23	23.1275	58.4363	<0.0001**
	Site x Year	82.4632	92	0.8963	2.2648	<0.0001**
Mn	Site	0.16919	4	0.04230	1.0712	0.369262
	Year	80.09333	23	3.48232	88.1858	<0.0001**
	Site x Year	8.09785	92	0.08802	2.2290	<0.0001**

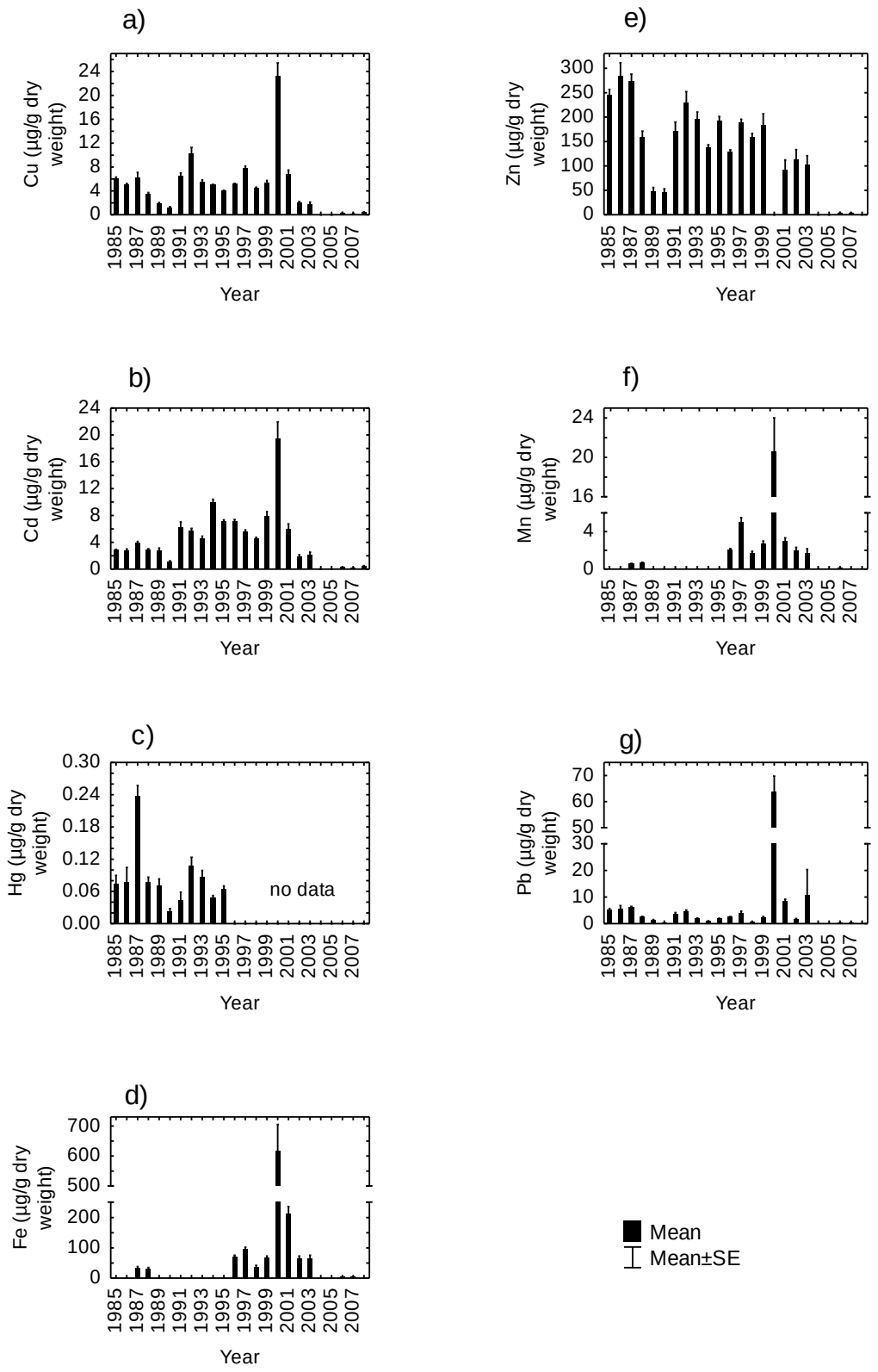

Figure 2-1. Mean (±SE) annual Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* for all sites combined from 1985 to 2008. No Hg data was collected from 1995-2008.

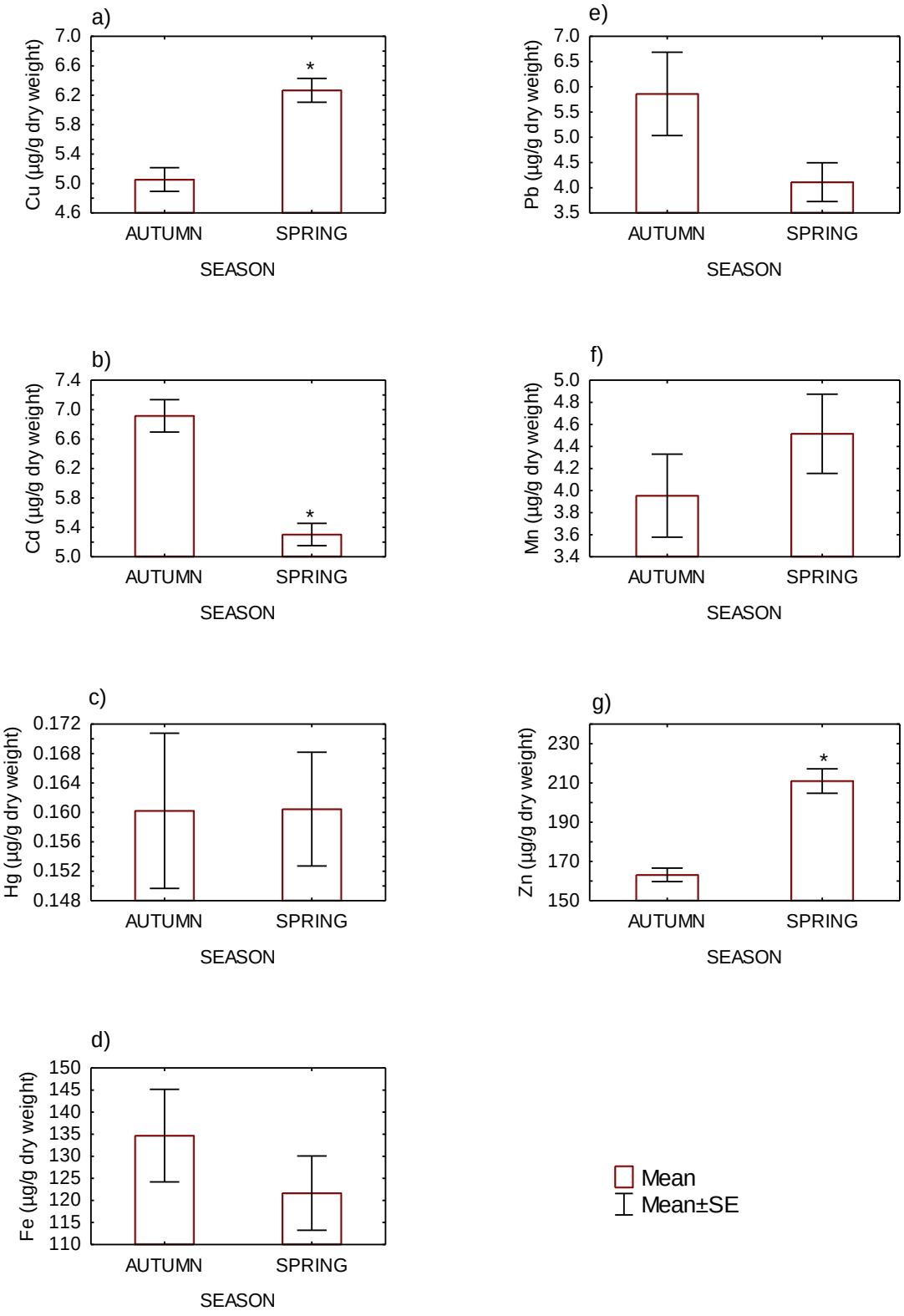

Figure 2-2. Mean (±SE) seasonal Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* measured at all sites from 1985 to 2008. * indicates significant differences using one way ANOVA ($p<0.05$).

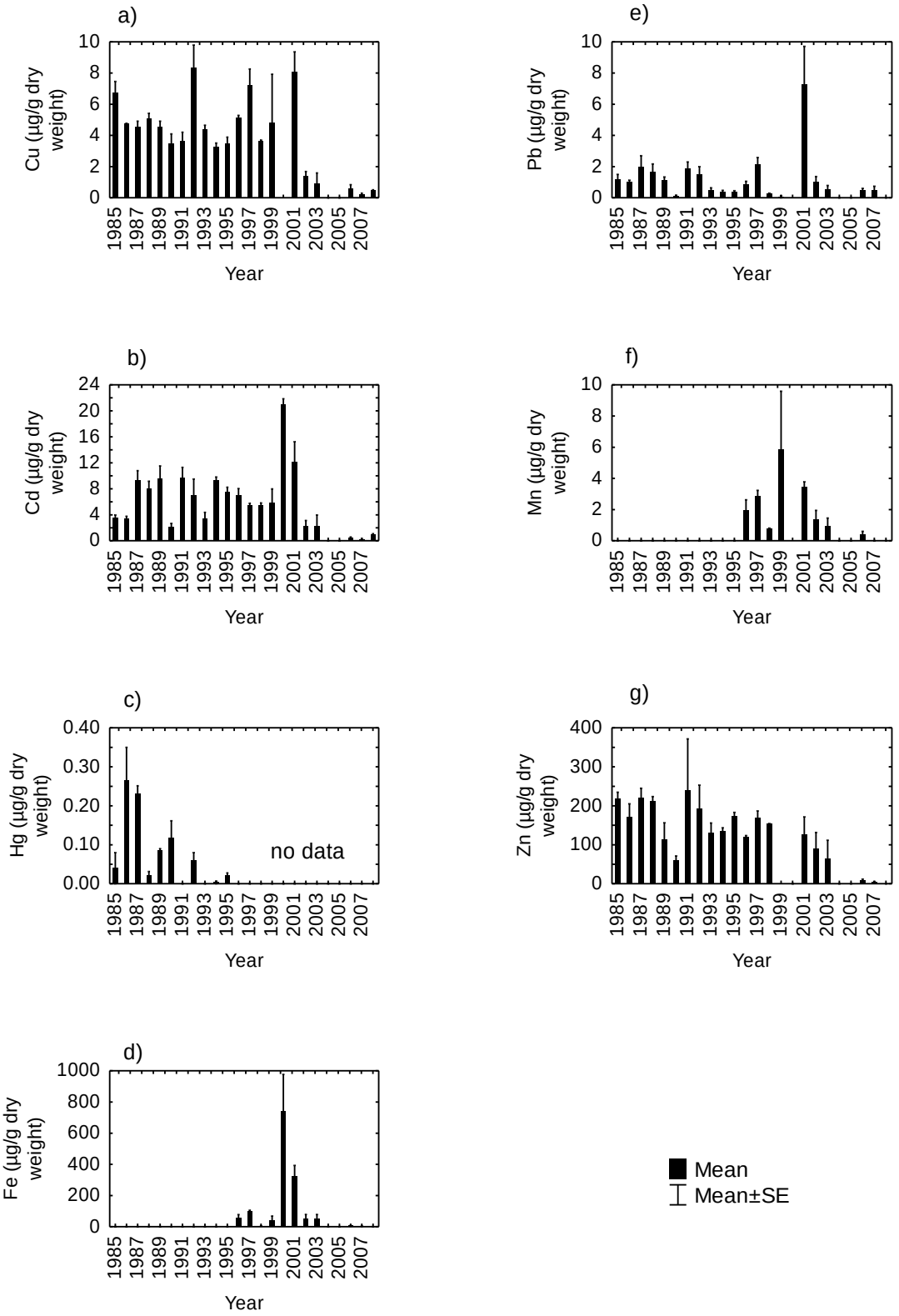

Figure 2-3. Mean (±SE) annual Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* for site 1 from 1985 to 2008. No Hg data was collected from 1995-2008.

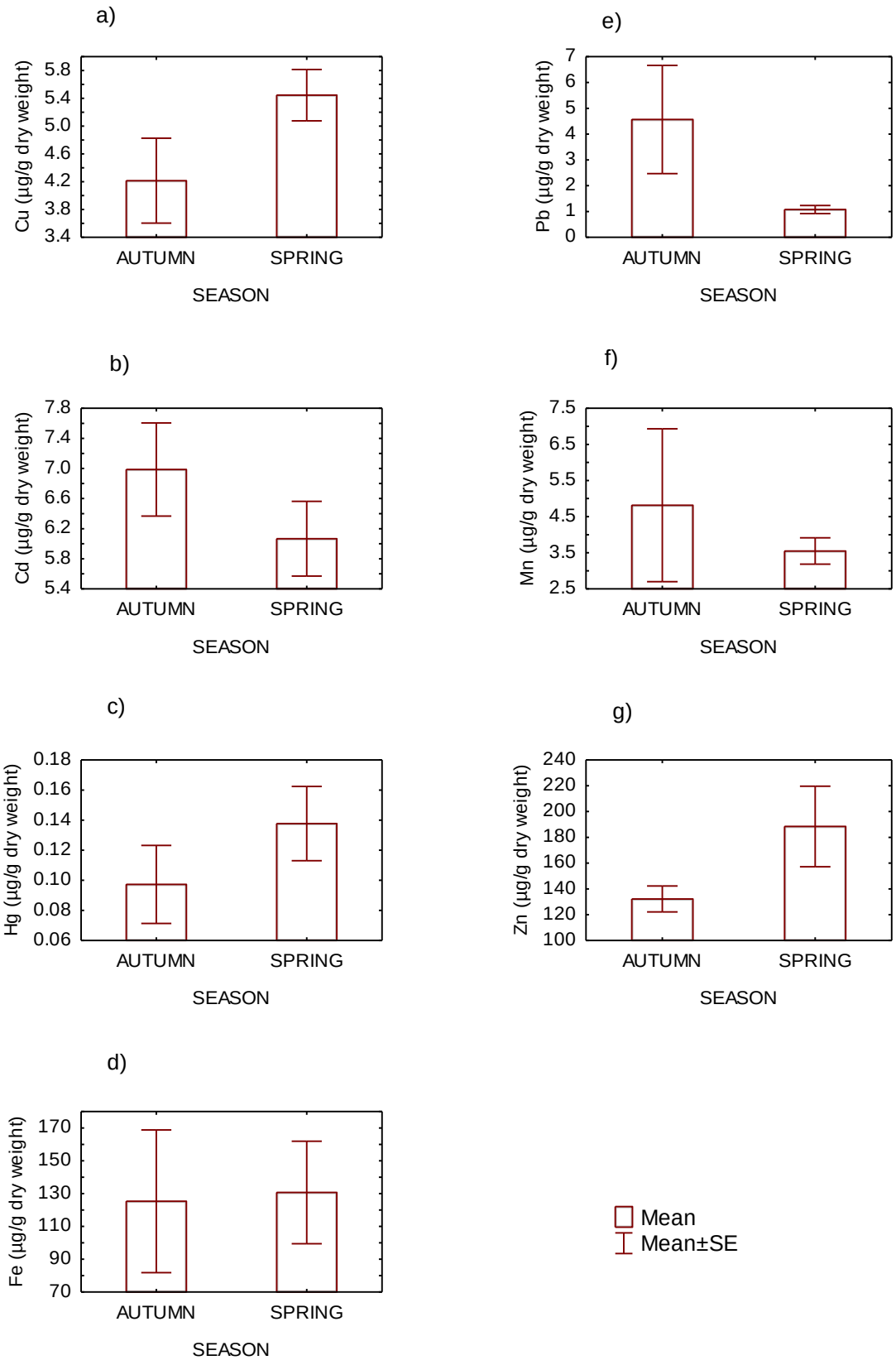

Figure 2-4. Mean (±SE) seasonal Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* measured at site 1 from 1985 to 2008. * indicates significant differences using one way ANOVA ($p<0.05$).

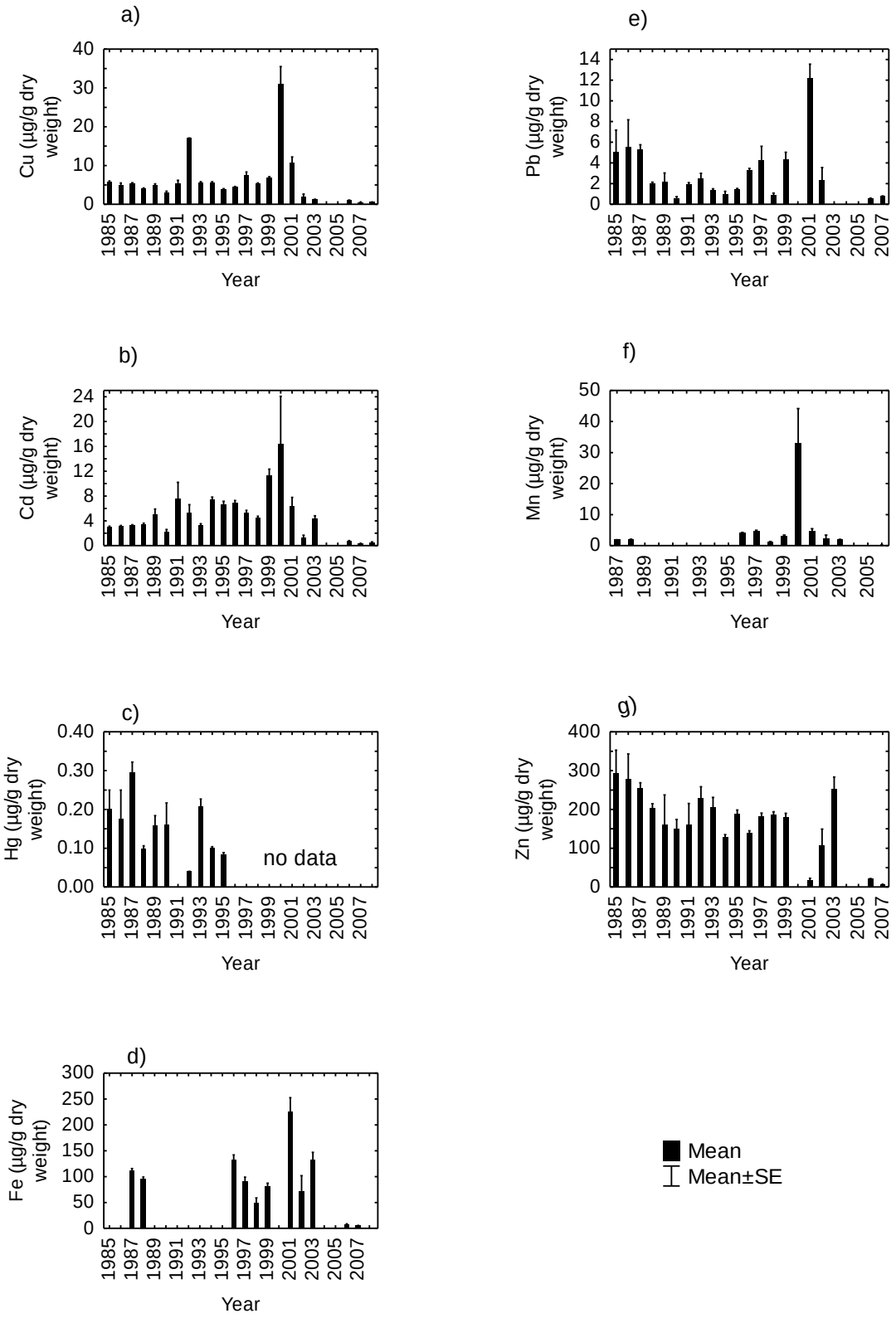

Figure 2-5. Mean (±SE) annual Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* for site 2 from 1985 to 2008. No Hg data was collected from 1995-2008.

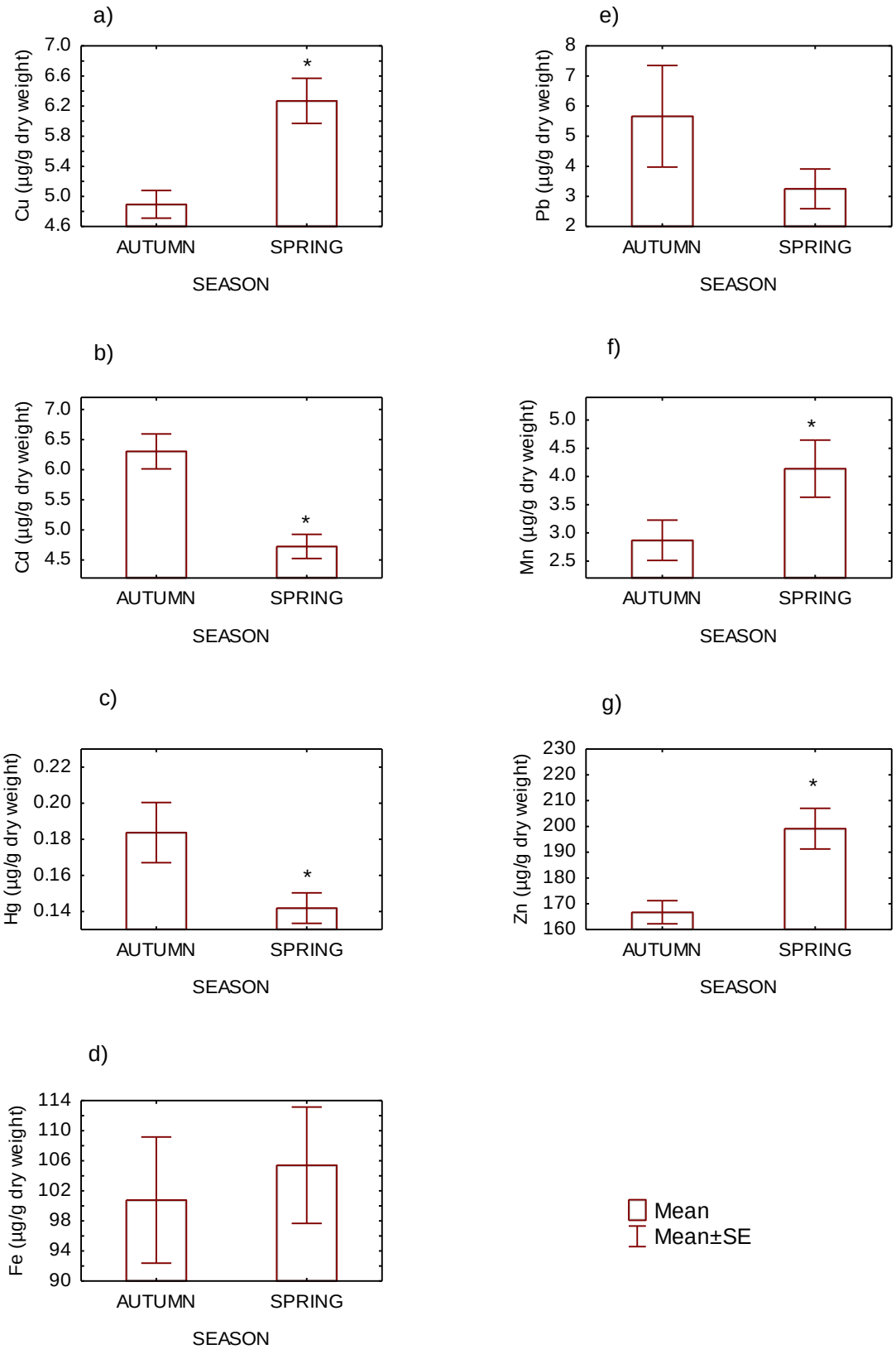

Figure 2-6. Mean (±SE) seasonal Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* measured at site 2 from 1985 to 2008. * indicates significant differences using one way ANOVA ($p<0.05$).

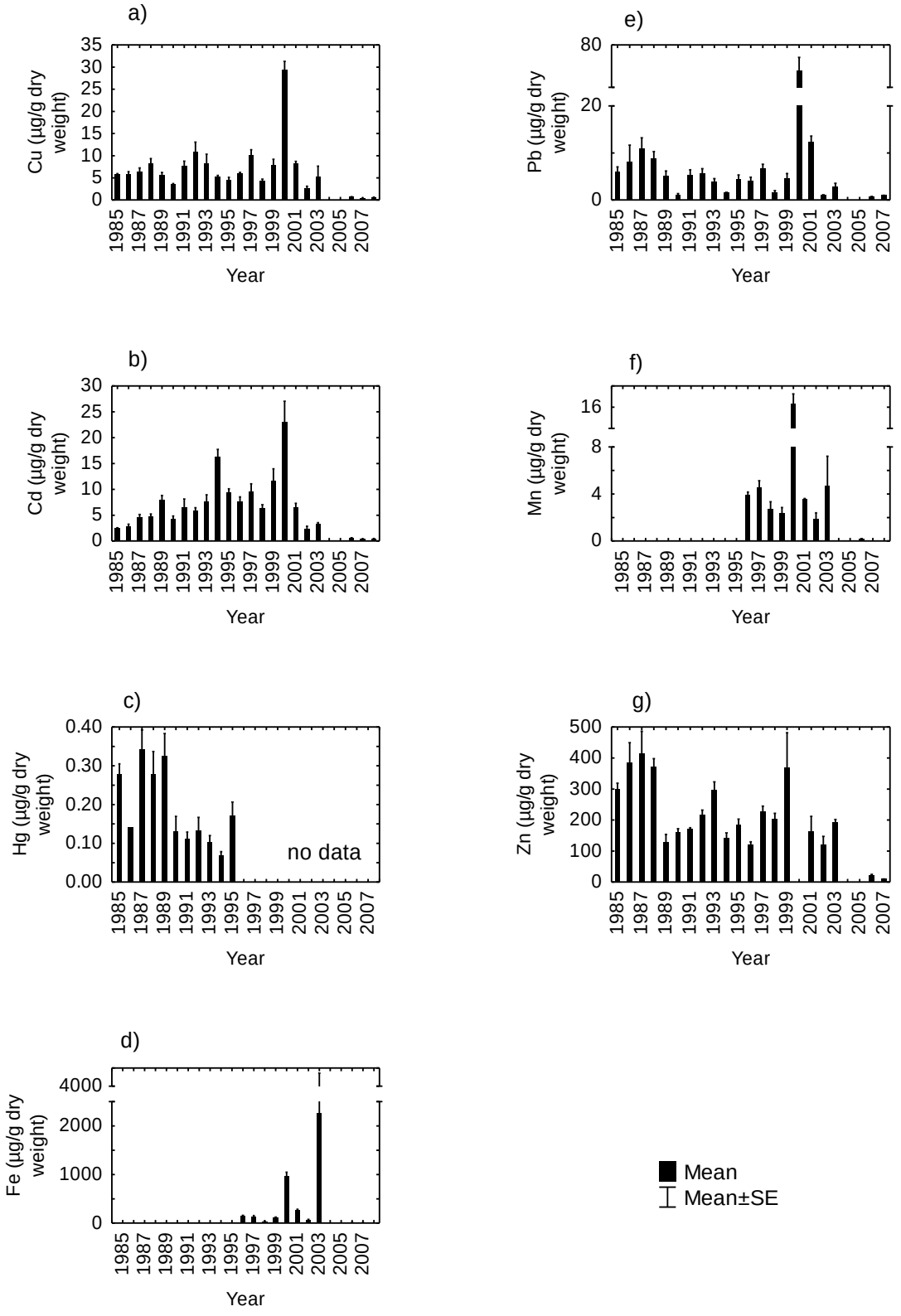

Figure 2-7. Mean (±SE) annual Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* for site 3 from 1985 to 2008. No Hg data was collected from 1995-2008.

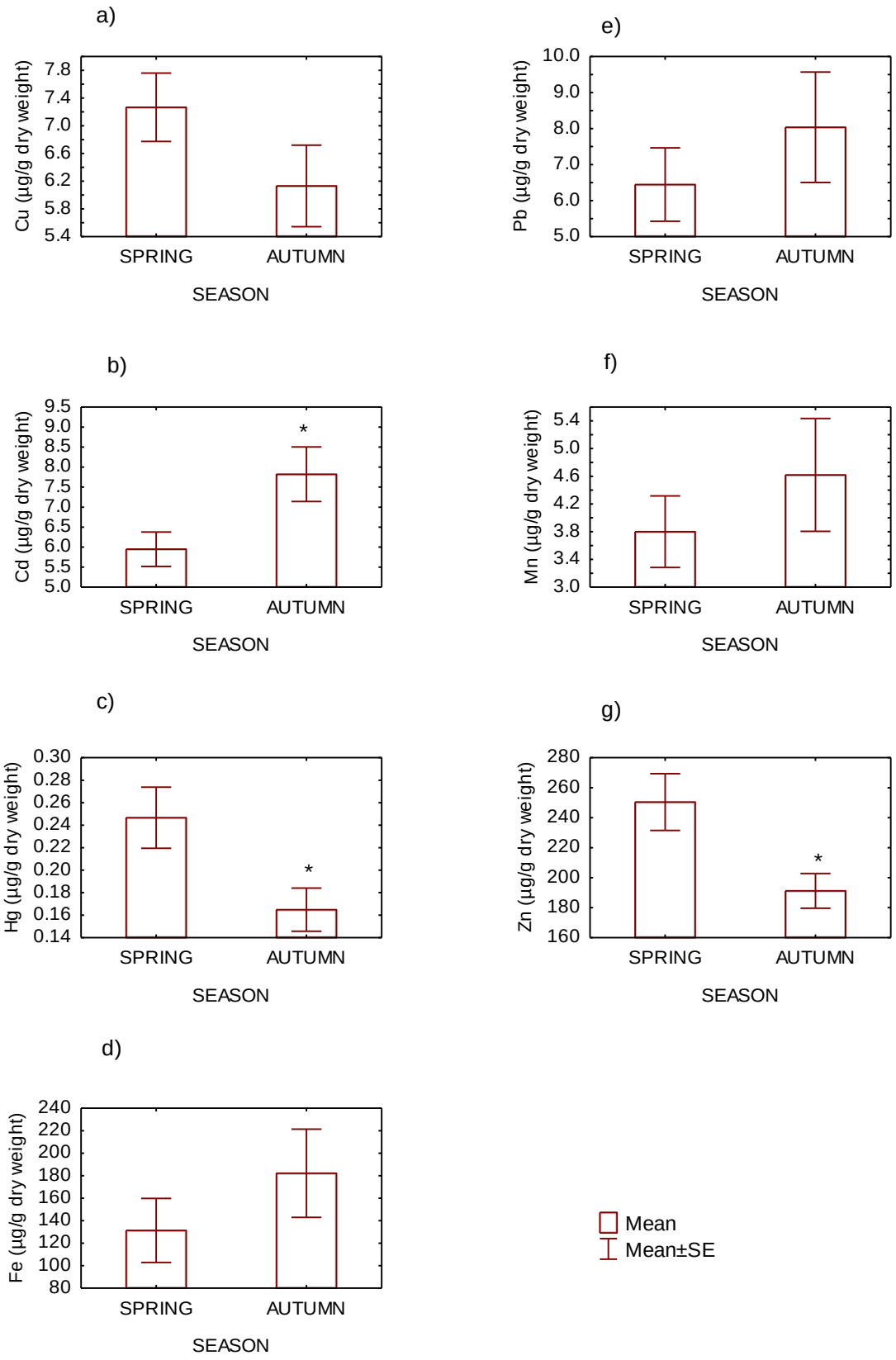

Figure 2-8. Mean (±SE) seasonal Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* measured at site 3 from 1985 to 2008. * indicates significant differences using one way ANOVA ($p<0.05$).

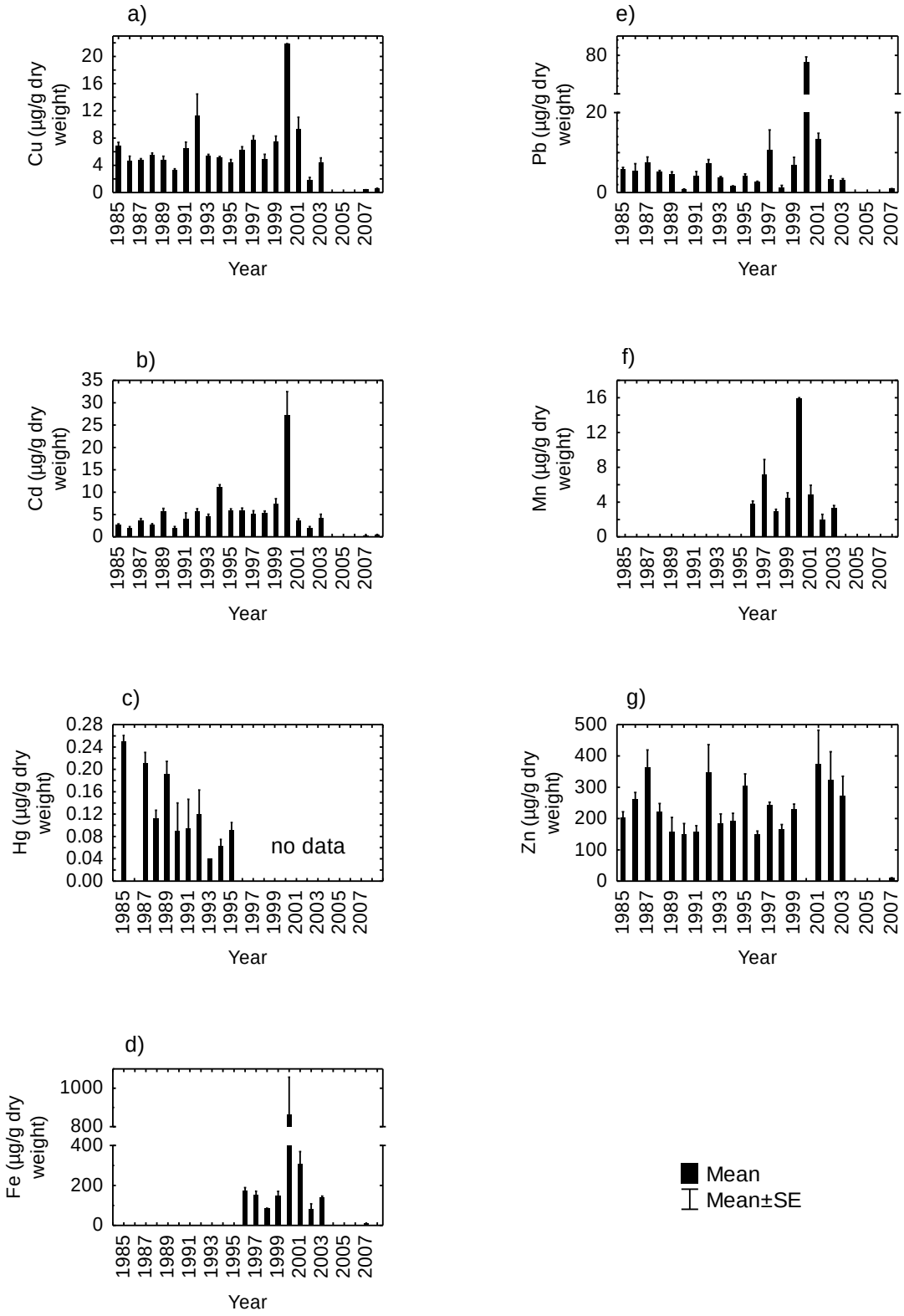

Figure 2-9. Mean (±SE) annual Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* for site 4 from 1985 to 2008. No Hg data was collected from 1995-2008..

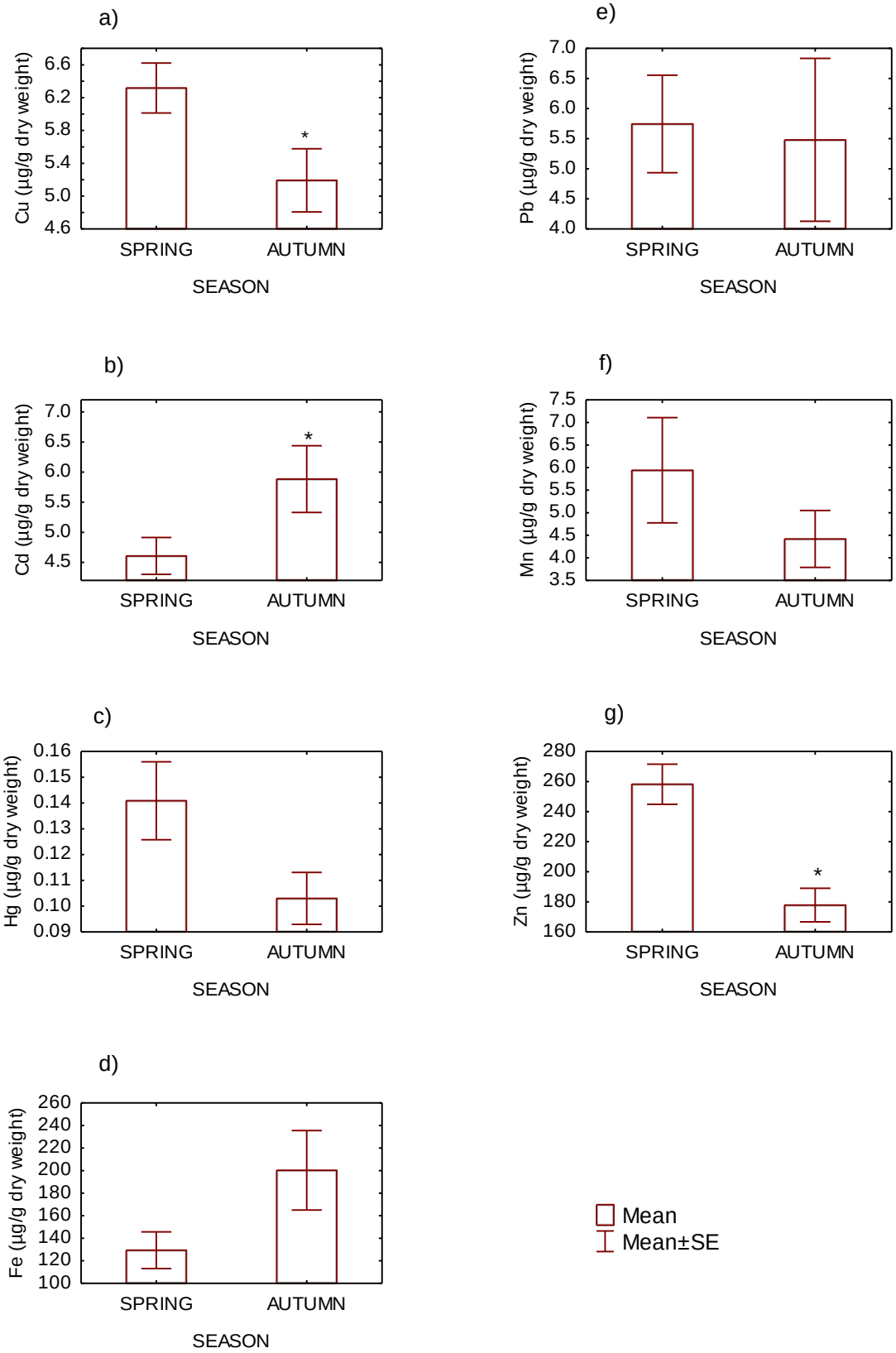

Figure 2-10. Mean (±SE) seasonal Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* measured at site 4 from 1985 to 2008. * indicates significant differences using one way ANOVA ($p<0.05$).

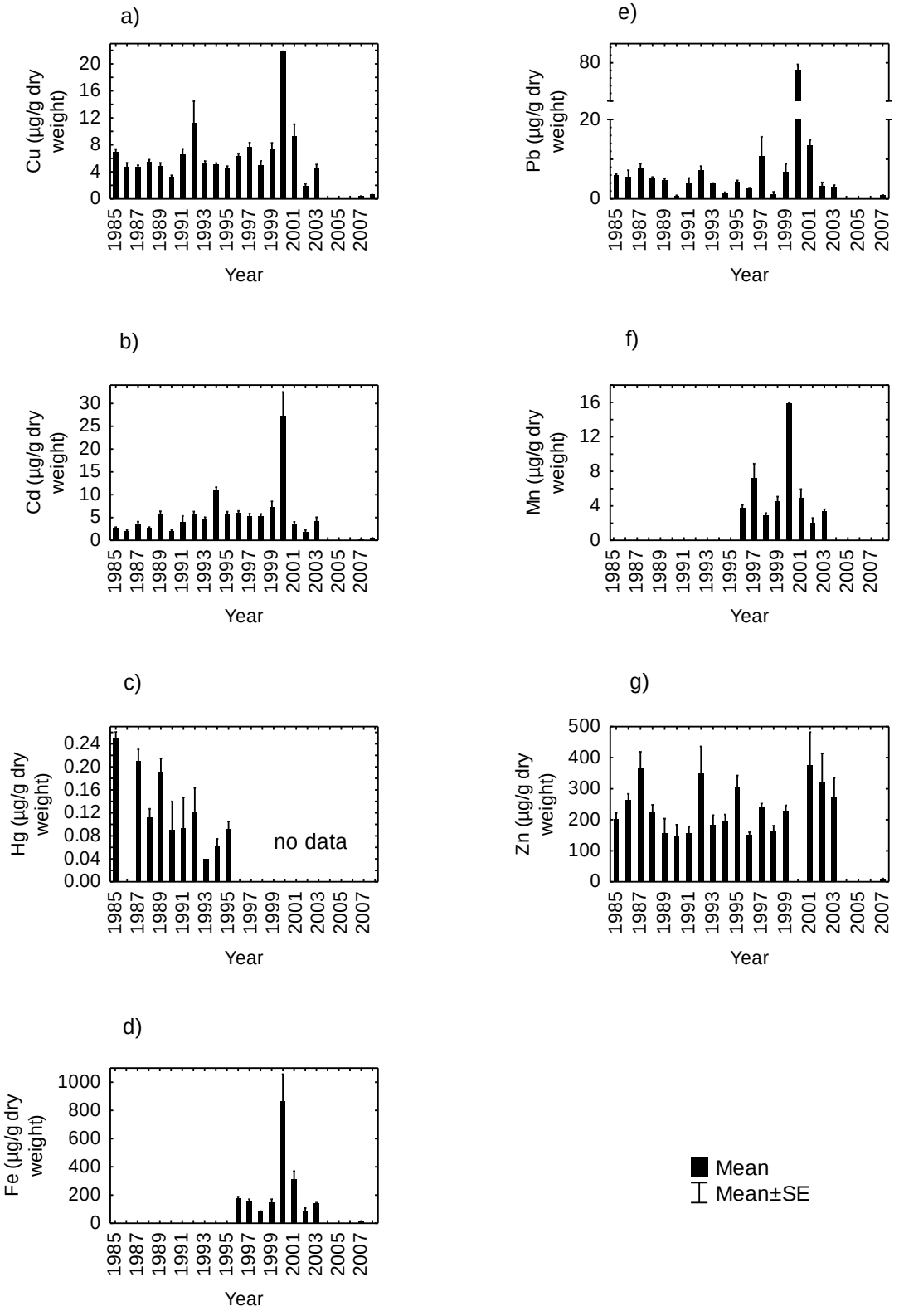

Figure 2-11. Mean (±SE) annual Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* for site 5 from 1985 to 2008. No Hg data was collected from 1995-2008.

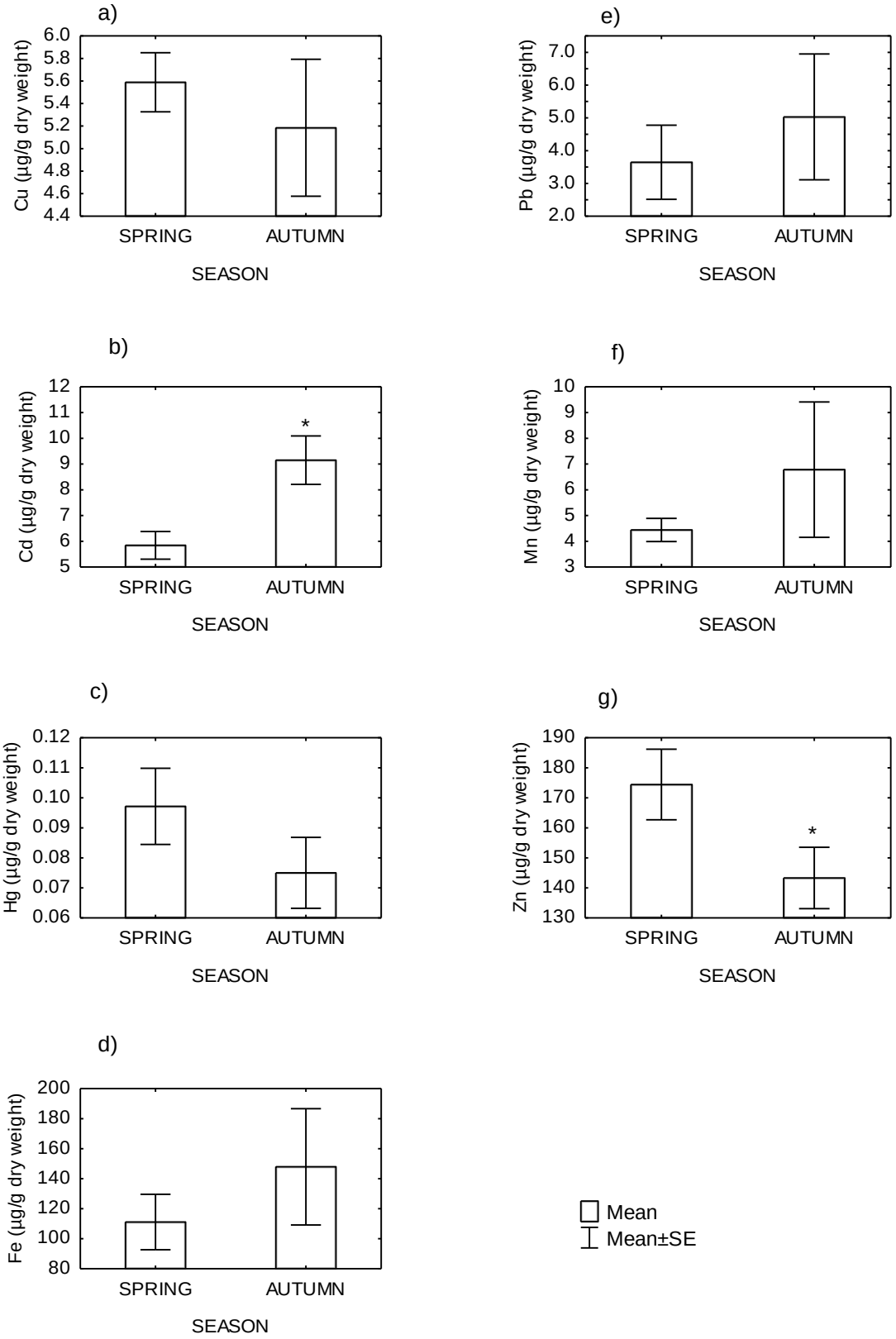

Figure 2-12. Mean (±SE) seasonal Cu (a), Cd (b), Hg (c), Fe (d), Pb (e), Mn (f) and Zn (g) (µg/g dry weight) concentrations in *M. galloprovincialis* measured at site 5 from 1985 to 2008. * indicates significant differences using one way ANOVA ($p<0.05$).

Chapter 3

METAL CONCENTRATIONS IN INTERTIDAL SEDIMENT AND WATERS OFF THE WEST COAST OF THE CAPE PENINSULA

3.1. Introduction

Pollution of the environment is the result of excessive release of chemicals into the system. The effects on organisms exposed to pollutants are often not evident until changes in the ecosystem have taken place. One way of determining whether compounds are having deleterious effects on the environment is to measure levels in the environment before their impact becomes evident. This can be accomplished by having a monitoring system that measures the level and types of compounds prevalent in the system. Of particular importance to monitoring effects of pollutants is that the compounds need to be ubiquitous. Metals are elements that occur naturally in the environment and are considered ideal to monitor pollution in sentinel organisms (Clark *et al.*, 1992). The source of metal pollution is mainly from anthropogenic sources due to agricultural activity, industrial efforts and an increase in urbanisation. Metals such as Cd, Cu, Ni, Pb and Zn tend to accumulate in aquatic environments due to their persistent nature (Clark *et al.*, 1992; Ankley *et al.*, 1996), in particular, within sediment layers (Sarki *et al.*, 1995).

Sediment can be considered to be a heterogeneous combination of particles of dissimilar compounds (such as detritus, inorganic particles, organic matter, plankton bacteria, etc). These compounds in turn comprise delicate and complicated inorganic and organic components (Martin *et al.*, 1987). According to El-Sammak and Aboul-Kassim (1999), analysing sediments can indicate the prevailing quality of a system and the pollution history of a particular area. The indication of the quality however is not a permanent one as metals are not necessarily fixed by the sediment but may be recycled via both biological and chemical mechanisms. According to Salomons and Förstner (1984), fixation and recycling can happen within the sediments as well as between sediment and the water column.

Metals in sediments are vital for the ecological functioning of aquatic biota and have greater concentrations in sediment than in the aquatic environment (Ünlü *et al.*, 2008), albeit that it impacts on both benthic and pelagic organisms. The majority of metals (such as Cu, Sn, Al and Cd) enter the marine environment via natural pathways such as erosion and volcanic activity (Clark *et al.*, 1992). Deleterious amounts of anthropogenic metals in the sediment can bioaccumulate within the organisms, which in turn can negatively impact both the natural ecosystem and human consumers of organisms within the ecosystem (such as predators of the benthic system). In some cases, concentrations of metals in the sediment are used as indicators of the health of that ecosystem (Ünlü *et al.*, 2008). Data on metal concentrations in sediment can also be used in areas where there are very low concentrations within the water column. Prevalence of metals in sediments is considered a better indicator than water given the continual flux of the aqueous part of the ecosystem (Sarki *et al.*, 1995).

Marine pollution has detrimental effects on the ecosystem and is increasingly becoming of great concern to most countries. The extent and impact of the effects is not clearly known. However, research on the sources and ecological effects that pollution is having on marine ecosystems is being done, particularly in coastal ecosystems (Salomons & Förstner, 1984). Metals are increasingly being introduced to the marine ecosystem from the atmosphere (Gou & Chou, 1991). These metals then settle and accumulate on the benthic sediment layer. Metals accumulate over time and can pose threats to the condition of that water column and health of benthic organisms as the effects of pollutants (such as metals) include change in genotype, which in turn affects gene pools of organisms (Kim *et al.*, 2003). It is therefore important to monitor both the sediment and aqueous environment, in particular sediments, as these form sinks for pollutants. This might not be practical in certain situations given the dynamic nature of the aquatic environment. Because of their accumulative nature, the elevated levels of metals in the water column can accumulate in the sediment and in turn can result in toxic environments for organisms inhabiting benthic areas (Salomons & Förstner, 1984).

The objective of this study was to measure the levels of metals in the intertidal waters along the west coast of the Cape Peninsula by measuring concentrations of metals in

the surface water and sediments and discussing the potential impacts these levels may have on the area.

3.2. Description of the study area and study sites

The study area was situated in the Western Cape, South Africa and included sites along the west coast of the Cape Peninsula, where the sites ranged from Scarborough to the south to Bloubergstrand, approximately 50 km to the north (Figure 3-1). The area comprises a largely rocky, mountainous feature dominated by the Table Mountain chain (van Herwerden & Bally, 1989). A series of mountain ranges extends from Table Mountain (1086 m), past the Constantiaberg (928 m) and Muizenberg (507 m) ranges to the Swartberg (678m), to the south of Simons Town. Historically, urban development has centered on the slopes of Table Mountain, initially starting around the safe anchorage of Table Bay, and then gradually spreading southwards, mainly along the eastern sides of the Table Mountain chain. According to van Herwerden and Bally (1989), the shoreline along the Cape Peninsula is dominated by rocky shores along the mountainous section of the Peninsula, interspersed with pocket beaches of sand or mixed sand and rock. The geology of the area consists of mainly the Malmesbury group with granite underlying most of the western part of the Peninsula (Glass, 1981).

The area falls within a Mediterranean-type climatic region, typified by winter rainfall from successional cold fronts from the west and dry southeasterly winds during the summer. Winter frontal systems cause north and westerly winds. The annual mean temperature in the region is 17°C (range ± 10°C). Because it is in a winter rainfall region, the area receives the bulk of its mean annual precipitation of between 500-700 mm mainly during the months of April to August (Shannon, 1985).

Five sampling sites along the west coast of the Cape Peninsula were chosen for this study. The coordinates for the five sites are indicated in Table 3-1. The sites selected were similar to those of the MWP sites in Chapter 2. Scarborough was the reference site (Figure 3-1) and is the southern-most sampling site situated in a coastal conservation village. The town is approximately 10 km north of the Cape Point Nature Reserve with a few houses at the foot of the mountain slope. The area comprises

sandy shores with outcrops of rocky shores. The geology is of the Malmesbury group and is representative of the rest of the sites. The sediment at Scarborough comprises rich deposits of calcium carbonate derived from shells of mussels and barnacles (Glass, 1981).

Hout Bay is a large U-shaped indentation along the west coast of the Cape Peninsula. The bay is approximately 2.5 km long and 2 km wide (Fricke *et al.*, 1979). A sandy beach is situated along the northern boundary with steep, rocky cliffs along the eastern boundary of the bay. A commercial fishing harbour is situated on the western side of the bay and sewerage is discharged close to this site. The beach is a popular recreational attraction for locals. The Palmiet River opens on the east side of the bay (Fricke *et al.*, 1979), with weak flows during summer months. Hout Bay harbor is situated at the western side of the bay. The sampling site is near an ablution facility and shopping centre. To the north of the site is a heavily populated informal settlement as well as farms (further to the north). The presence of the informal settlement, fish factories, fishing vessel maintenance sites may be regarded as potential pollution impacts and hence monitoring this area is important.

Green Point is situated at the northern most end of the Sea Point promenade and is within a kilometer of the Cape Town Soccer Stadium. The area is intensively utilised for beach recreation and stormwater discharge (Quick & Roberts, 1992). According to Quick and Roberts (1992), pollutants discharged into the area consisted mainly of phosphorous, nitrogen and zinc.

The fourth site at Milnerton is situated close to the outlet of an outfall pipe close to the mouth of the Diep River and the Salt River (Quick & Roberts, 1992). The site is within a couple of kilometers of Table Bay harbour, one of the busiest ports in South Africa. It is adjacent to a suite of beachfront hotels and is situated at the shoreward end of an industrial area (Paarden Island). Potential sources of pollution are from industrial activities in Paarden Island, a refinery approximately 15 km upstream of Diep River as well as runoff from the neighbouring urban areas. The coastline along Table Bay has regressed up to 42 m over the past 50 years due to weathering and sediment movements, but the rate of regression has decreased (Quick & Roberts, 1992).

Bloubergstrand is an urbanized area with a rocky shoreline situated approximately 20 kilometers north of Cape Town. The intertidal zone has rocky shores that are exposed and experiences strong wave action and intense upwelling (Orren *et al.*, 1980). The site is north of a water-contact recreation area and is considered sufficiently far from areas to be influenced by pollution (Orren *et al.*, 1980). There has been intense urban development in the area, to which the impact on the area is not known.

3.3. Materials and Methods

3.3.1. Sediment sampling

Sediment samples were collected seasonally between March 2010 and March 2011, during the months of March 2010 (autumn 2010), July (winter 2010), September (spring 2010), January (summer 2010), and March 2011 (autumn 2011). Seasonal samples were collected to ascertain temporal trends in metal concentrations in the sediment. All samples were collected at spring 2010 low tide, with sampling always commencing at the southern-most site (Scarborough). Most sampling commenced at approximately 09h00. Samples were collected at five sites with Scarborough considered to be the reference site as it was situated at the southern-most position of the sites and away from industrial, agricultural and heavy residential sites.

Sediment samples were collected using a small plastic scoop within 10 m of mussel sampling. Six replicates were collected at each site at *ad hoc* intervals. The sediments were placed into a plastic 300 mL jar and placed in cooler boxes whilst being transported to the laboratory. Once in the laboratory, the samples were frozen at -20 °C until analysis was done.

3.3.2. Water sampling

Water samples were collected at the same area as sediment samples. The water samples were collected at the same low tide, within 10 m of mussel sampling and approximately 40 cm above the sediment. Six replicate water samples were taken

using a 200 mL jug and placing approximately 50 mL of sea water into a 300 mL plastic container. The water samples were then placed in cooler boxes whilst being transported to the laboratory. Once in the laboratory, the samples were frozen until analysis was done. Water from the sampling sites was placed in a five litre bucket and pH and temperature measurements taken at the site. The pH was measured using a Hanna Hi 9810 portable pH/EC/TDS meter and temperature was determined using a standard thermometer.

3.3.3. Metal analysis

Metal analysis was done according to the method of Odendaal and Reinecke (1999). Frozen sediment samples were defrosted, whereafter they were oven dried for 48 hrs at 60° C in a Memmert drying oven. Sediment was ground with a mortar and pestle and subsamples (± 0.2 g) used for metal analysis. Ground sediment aliquots and defrosted water samples (5 mL water) were digested using 10 mL of nitric acid (HNO_3). Samples were then heated to 40 °C in a Grant UBD heating block for one hour, thereafter to 120 °C for 3 hours. The digestates were allowed to cool and then filtered through Whatman No. 6 filter paper and then through 0.45 µm membrane micro-filter (Millipore) paper using a syringe. Samples were then placed in plastic centrifuge tubes containing 5 mL digestate and 10 mL distilled water and stored in a refrigerator until further analysis was done at the University of Stellenbosch.

A blank accompanied all samples when analysis of samples took place. The concentrations of manganese (Mn), Iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) were analysed, with 7-8 replicates being done for each metal using an Agilent 7700 Inductively Coupled Plasma – Mass Spectrophotometer (ICP – MS). Detection limits for all metals analysed was 0.1 ppb. Concentrations of metals are presented as µg/g dry weight for sediments and as µg/L for water. Analytical standards (Standard Reference Material) were used for all metals to determine the analytical limits. All metal concentrations were within 2% of the reference material.

3.3.4. Statistical analysis

All calculations and data analysis were done using Statistica 10 (Statsoft). One way ANOVA was used to determine whether there were seasonal and spatial differences in mean metal concentrations. The data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests respectively, prior to post hoc comparisons. Data that did not meet the requirements were $\log_{10}$ transformed. Post-hoc comparisons were made using the Tukey Honest Significant Difference (HSD) test to determine statistically significant data ($p<0.05$). The Dunnett post hoc test was used to determine significant differences between Hout Bay, Green Point, Milnerton, Bloubergstrand and Scarborough (control site). Spearman Rank correlations were used to determine relationship between metal concentrations in water and sediment at both spatial and temporal levels.

To quantify the magnitude of pollution by metals, the contamination factor (CF) was determined. The CF is an indicator by which factor the background concentration is exceeded at a site and is calculated as follows:

$$CF = \frac{Metal\ concentration\ in\ sediments}{Background\ levels\ for\ shallow\ marine\ sediments}$$

(El-Sammak & Aboul-Kassim, 1999).

The Pollution Load Index (PLI) is used as a means to determine the mutual pollution effects at different sites as determined by the different metals (El-Sammak & Aboul-Kassim, 1999). For this study, the PLI was calculated using the equation of El-Sammak & Aboul-Kassim (1999):

$$PLI = \sqrt[6]{(CF_{Fe})\,(CF_{Mn})\,(CF_{Cu})\,(CF_{Zn})\,(CF_{Cd})\,(CF_{Pb})}$$

According to El-Sammak & Aboul-Kassim (1999), the higher the PLI, the higher the stress from pollution.

3.4. Results

3.4.1. Water

3.4.1.1. Temperature

The coastal sea water temperatures recorded during the study period were indicative of the prevailing oceanic conditions. The temperatures ranged between 14 and 16 °C (Figure 3-2). The mean temperature for the study period was 16 ± 1.34 °C. The lowest mean temperatures were recorded during winter 2010 15 ± 0.79 °C, followed by spring 2010 (15.5 ± 0.85 °C) with autumn 2010 having the highest mean temperatures (17 ± 0.85 °C). There were no significant differences in temperature between sites ($p>0.05$). There were however significant differences in temperature between winter 2010 and autumn 2010 as well as spring 2010 and autumn 2010 ($p<0.005$).

3.4.1.2. pH

The pH values for the study area ranged between 7.03 and 8.14 (Figure 3-3). For most sites, the pH increased from autumn 2010 to winter 2010 and then decreased during spring 2010 (except for Green Point). The mean pH recorded for the study period was 7.76 ± 0.17. The Tukey HSD post hoc test of one-way ANOVA indicated that there were significant differences in pH between sites one and four, three and four as well as four and five ($p<0.005$). There was also a significant difference between winter 2010 and autumn 2010 pH ($p<0.005$).

3.4.1.3. Metal concentrations in water

3.4.1.3.1. Iron

The mean Fe concentration for the entire study period was 3.27 ± 23.6 µg/L. Iron concentrations were lowest at Scarborough, Hout Bay and Green Point (Figure 3-4). High concentrations of Fe were recorded during winter 2010 at Milnerton (4.17 µg/L)

and Bloubergstrand (69.53 µg/L). Only Fe concentrations at Milnerton and Bloubergstrand were significantly higher than the other sites sampled.

3.4.1.3.2. Manganese

Manganese concentrations in the coastal waters of the Cape Peninsula were very low at Scarborough, Hout Bay and Green Point, with a mean concentration of 0.05 ± 0.32 µg/L recorded for the study period (Figure 3-5). There were only two relatively high mean concentrations of Mn, recorded during winter 2010 at Milnerton (0.127 µg/L) and Bloubergstrand (1.01 µg/L). There was only a significant difference between spring 2010 and winter 2010 Mn concentrations ($p<0.05$). The seasonal and spatial Mn patterns were very similar to those of Fe concentrations, with Milnerton and Bloubergstrand Mn concentrations being significantly higher than the other sites sampled.

3.4.1.3.3. Copper

The Cu concentrations recorded in coastal waters of the Cape Peninsula were the lowest of all the water metals analysed (Figure 3-6). The mean Cu concentrations recorded were 0.002 ± 0.009 µg/L. The highly variable concentrations of Cu indicated that only Cu in autumn 2011 at Hout Bay and autumn 2011 at Bloubergstrand were significantly higher than the other sites sampled. There were no significant differences between sites.

3.4.1.3.4. Zinc

The Zn concentrations in the coastal waters were very low (Figure 3-7). The mean Zn concentration for the study period was 0.11 ± 0.54 µg/L. There was only one significant peak in Zn recorded at Milnerton during winter 2010. There were no significant differences between sites ($p>0.05$). Although not significant, there were higher Zn concentrations at Bloubergstrand than Scarborough, Hout Bay and Green Point.

3.4.1.3.5. Cadmium

The mean Cd concentration for the study period was 0.004 ± 0.01 µg/L (Figure 3-8). Only during autumn 2010 at Green Point (0.015 µg/L) and winter 2010 at Milnerton (0.0084 µg/L) were Cd concentrations significantly higher than the other sites sampled. There were no significant spatial Cd trends observed during the study period.

3.4.1.3.6. Lead

The mean Pb concentrations in coastal waters of the five sampling sites were 0.003 ± 0.01 µg/L (Figure 3-9). Lead values at Bloubergstrand were significantly higher than Scarborough ($p<0.05$). Significantly higher concentrations of Pb were recorded at Milnerton in winter 2010 (0.017 µg/L) and during winter 2010 at Bloubergstrand (0.04 µg/L) than the rest of the sites sampled. There were no significant differences between sites observed in the coastal waters.

3.4.2. Sediment

3.4.2.1.1. Metal concentrations in sediments

3.4.2.1.1.1. Iron

The Fe concentrations in sediments from different sites were grouped per season and site (Figure 3-10). The mean Fe concentration was 835.6851 ± 706.61 µg/g dry weight with the highest value of 7041.11 µg/g recorded at Green Point during summer 2010. Mean Fe values were lowest at Scarborough (<0.1 µg/g) and highest during winter 2010 at Green Point (2409 µg/g). Tukey's HSD test indicated significant differences between Hout Bay and Green Point as well as between Milnerton and Bloubergstrand ($p<0.05$). There were no significant differences in seasonal Fe

concentrations. However all the sites had significantly higher Fe concentrations than Scarborough.

3.4.2.1.2. Manganese

Manganese concentrations were relatively low for the study period (Figure 3-11) and had a mean concentration of 4.66 µg/g dry weight. The highest Mn values recorded during the study period were at Green Point (20.00 ± 7.52 µg/g) during summer 2010 with the highest readings for that season being 46.71 µg/g. At Scarborough, Green Point and Milnerton, the Mn values increased from autumn 2010 to autumn 2011. The Tukey HSD test indicated significant differences in Mn concentrations between all the sites ($p<0.05$).

3.4.2.1.3. Copper

The Cu concentrations in sediment for the study period were relatively low, with most sites recording less than 5µg/g dry weight (Figure 3-12). The mean Cu concentration in sediments for all the sites was 2.73 ± 7.14 µg/g with the highest concentration recorded at Hout Bay in spring 2010 (54.49 µg/g). The Tukey HSD test indicated that Cu concentrations were significantly higher during spring 2010 at Scarborough, Hout Bay and Green Point, during autumn 2011 at Hout Bay and Green Point as well as during summer 2010 at Green Point ($p< 0.05$). There Cu concentrations were significantly higher at Scarborough and Green Point than Milnerton and Bloubergstrand.

3.4.2.1.4. Zinc

The Zn concentrations along the west coast of the Cape Peninsula had low concentrations during the study period. The mean Zn concentrations in sediment were 14.26 ± 5.5 µg/g dry weight (Fig 3-13). Highest Zn concentrations were recorded at Bloubergstrand during winter 2010 (387.52 µg/g). There were relatively low Zn concentrations at Scarborough, Hout Bay and Milnerton (<20 µg/g). Only

Bloubergstrand had significantly higher Zn concentrations ($p<0.05$) during winter 2010 than the other seasons sampled. There were no significant differences between sites.

3.4.2.1.5. Cadmium

Cadmium had the lowest mean concentrations of all the metals analysed in sediment and had a mean value of 0.29 ± 1.19 µg/g dry weight (Figure 3-14). There were only two seasons with significantly higher mean Cd values which were recorded during autumn 2010 at Green Point and during winter 2010 at Bloubergstrand. There were no significant differences in Cd concentrations between the sites.

3.4.2.1.6. Lead

Mean Pb concentration in sediments for the study period was 1.89 ± 1.51 µg/g dry weight (Figure 3-15). The highest Pb values were recorded at Green Point (12.831 µg/g dry weight) during summer 2010. Scarborough had the lowest mean Pb sediment concentrations (0.189 µg/g). Lead concentrations at Hout Bay, Green Point and Milnerton were significantly higher than the mean ($p<0.005$). There were significantly higher Pb concentrations recorded during autumn 2010 at Scarborough, Hout Bay and Green Point as well as during autumn 2011 at Bloubergstrand. The Pb concentrations in sediment of all the sites sampled were higher than Scarborough with the concentrations at Hout Bay, Green Point and Milnerton being significantly higher than the mean.

3.4.2.2. Correlations between water and sediment concentrations

The Pearson's product moment correlations between sediment and water metal concentrations are presented in Table 3-2. Generally, there were no significant correlations or trends between sediment and water samples when all metal data were combined. However, there was a significantly strong positive correlation ($r>0.9$) between sediment and water Cd during autumn 2010 (Table 3-2). The remaining

metals did not display any correlations during autumn 2010. Winter 2010 and spring 2010 correlations were negative for all metals, but these were not significant trends.

There were no significant correlations between metals at Scarborough. At Hout Bay, there were weak positive correlations ($0.6<r>0.9$) between sediment and water Fe, Mn and Pb. Metal concentrations at Green Point also had a significantly weak positive correlations between Fe, Mn and Cd sediment and water metals ($p<0.05$). At Milnerton, there were weak positive correlations in Cd sediment and water concentrations but poor positive correlations ($r<0.6$) for Mn, Fe and Pb. Metal concentrations at Bloubergstrand had poor positive correlations between sediment and water Zn, Cd and Pb concentrations.

3.4.3. Contamination factors and Pollution load indices

The contamination factor (CF) was calculated for each metal and categorised per season and per site. The calculations were based on background levels previously reported by Hennig (1985) per site (Table 3-3). Sediment CF is presented in Table 3-4. The background measurements of 1977 (Table 3-3) suggest that the area was in a pristine condition 40 years ago. Generally, the present study indicated that, per season, CF's were lowest in autumn 2010 (3.09 in 2010 and 2.58 in 2011), increased during winter 2010 (20.43), decreased towards spring 2010 (3.54) and then increased in summer 2010 (4.14).

During autumn 2010, Scarborough had the lowest CF for all metals combined. The order of mean seasonal CF for the different sites were autumn 2010: Hout Bay > Bloubergstrand > Green Point > Milnerton > Scarborough; winter 2010: Bloubergstrand > Milnerton > Hout Bay > Green Point > Scarborough; spring 2010: Hout Bay > Scarborough > Green Point > Bloubergstrand > Milnerton; summer 2010: Bloubergstrand > Milnerton > Green Point > Hout Bay > Scarborough; autumn 2011: Bloubergstrand > Hout Bay > Green Point > Scarborough > Milnerton. Scarborough had the lowest CF during autumn 2010 and winter 2010 but had the second highest CF during spring 2010. On a spatial scale, irrespective of season, the CF categorised from highest to lowest were: Bloubergstrand (34.44) > Hout Bay (6.24) > Green Point (2.34) > Milnerton (1.03) > Scarborough (0.854). This result suggests that

Scarborough was the least affected by pollution over the past 40 years compared to the other sites investigated in this study.

Pollution Load Index (PLI) is a method used to determine the mutual pollution effect at different sites (El-Sammak & Aboul Kassim, 1999). Table 3-5 presents the PLI for the five sites investigated along the west coast of the Cape Peninsula and indicates that pollution loads increased from autumn 2010, winter 2010 through to spring 2010, decreased in summer 2010 and then increased in autumn 2011. Categorization of PLI from highest to lowest per site was: Hout Bay > Bloubergstrand > Green Point > Milnerton > Scarborough. This result further indicates that Scarborough was least affected by pollution. The mean PLI results reported here are lower than that reported by El-Sammak & Aboul Kassim (1999) where an average of 1.279 was recorded for all the sites that they sampled. The mean PLI recorded for the five sites was 0.9808 combined and 0.997 for the five seasons sampled. El-Sammak & Aboul Kassim (1999) reported that sites that had PLI > 1.2 were considered to be affected by pollutants. Given that neither site nor season PLI values higher than 1, it is suggested that none of the sites samples were negatively affected by exposure to metals and not be considered polluted based on PLI values. This postulation however, needs further investigation as the degree of pollution is influenced by the type, source and quantity of pollutants.

3.5. Discussion

The City of Cape Town is situated at the southern tip of Africa and regarded as one of the most beautiful cities in the world. It was elected the best city in Africa in 2004, nominated as the world leading tourist destination in the 2005 world travel awards and was selected one of the world's top eight creative destination cities in 2002 (Anon, 2008). Cape Town and the Western Cape is also renowned for its high species richness, high beta and gamma diversity and high endemicity (Rebelo *et al.*, 2011) and as such is considered a world heritage site and biodiversity hotspot.

The area is ecologically important and for its condition to be pristine, it needs to be maintained and the effects of anthropogenic influences need to be minimal. This might prove difficult given that it is a rapidly developing urban hub of the country with

a population of 3.2 million people. The economic development is an important aspect to consider in maintaining its condition as unemployment could lead to social problems in coastal communities (including those in the study area such as Hout Bay). Although the GDP stands at R105 billion, the unemployment rate is 23% (Anon, 2008). The high unemployment rate could exasperate the pressure on the coastal environment as poor social structure and slow economic growth could have negative impacts on the status of coastal environments (Nahman & Godfrey, 2010).

The sources of marine pollution are varied but stems from the following sources: land-based disposal (domestic and industrial waste in solid and liquid forms); atmospheric sources; ship-borne pollutants; offshore oilwells and installations; pollution from explorations and exploitation of the sea; radioactivity; disposal of military materials, weapons as well as dumping at sea (Dittke, 2000). According to this author, the effects of the pollutants are numerous and varied. Of these various pollutants, metals have the ability to induce detrimental effects on marine organisms (Kennish, 1997; Shulkin *et al.*, 2003). This in turn could have negative consequences for the ecological functioning of the system. Furthermore, it is likely to have negative consequences for the economic development of the area. Since Cape Town is both ecological and economically valuable, knowledge about the conditions of the coastal area is pertinent and could likely influence the management strategies of the area. The objective of this study was to determine the health of the Cape Peninsula coastal waters by evaluating the concentrations of metals (Fe, Mn, Cu, Zn, Cd and Pb) in sediment and surface waters at five sites, collected seasonally from autumn 2010 to autumn 2011.

Metal concentrations in sediment sampled from Scarborough, Hout Bay, Green Point, Milnerton and Bloubergstrand were compared to the Canadian Sediment Quality Guidelines (CSQG) for the protection of aquatic life (CCME, 2001) as no sediment quality guidelines exist for South Africa. The guidelines are as follows: cadmium (0.6 mg/kg), copper (35.7 mg/kg), lead (35 mg/kg) and zinc (123 mg/kg). The CSQG does not have guidelines for iron and manganese. None of the metal concentrations at any of the sites were above the CSQG, except for zinc at Bloubergstrand during winter 2010 (387.52 µg/g). The high Zn level in the sediment at Bloubergstrand is discussed below.

Iron is the fourth most abundant element in the earth's crust and may be present in natural waters in varying quantities depending on the geology of the area and other chemical properties of the water body (Duffus, 1980). Ferric salts are insoluble in oxygenated waters, and hence iron concentrations are usually low in the water column. Iron is an essential micronutrient for all organisms, and is required in the enzymatic pathways of chlorophyll and protein synthesis, and in the respiratory enzymes of all organisms (Duffus, 1980). It also forms a basic component of haeme-containing respiratory pigments (for example, haemoglobin), catalyses, cytochromes and peroxidases (WHO, 1982). Under certain conditions of restricted availability of iron, photosynthetic productivity may be limited (Wright & Welbourne, 2002).

Iron is naturally released into the environment from weathering of sulphide ores (pyrite, FeS) and igneous, sedimentary and metamorphic rocks (Duffus, 1980). Leaching from sandstones releases iron oxides and iron hydroxides to the environment (Wright and Welbourne, 2002). Iron is also released into the environment by human activities, mainly from sewage, landfill leachates and the corrosion of iron and steel (Duffus, 1980; Wright and Welbourne, 2002). Various industries that also use iron in their processes, or in their products, include: the chloro-alkali industry; the household chemical industry; the fungicide industry; the petro-chemical industry (Duffus, 1980). According to Wright and Welbourne (2002), the water quality guideline for Fe in water is 0.3 µg/L for aquatic life.

Iron has the potential to have high concentrations in the natural environment (Giarratano *et al.*, 2010). Changes and sources of Fe concentrations for sites of the present study are related to varying contributions of the Table Mountain chain, with geological inputs from the Malmesbury group (Glass, 1981). Another source of Fe, and arguably the major contributor is the runoff from land, but from natural sources. According to Rebelo (2011), vegetation within Cape Town is strongly tied to geological composition and has been used to determine vegetation types. Fynbos is rich in Fe (up to 1136 µg/g in proteoid fynbos communities) (Mustart & Cowling, 1993) and this natural source of Fe is considered the major contributor to the elevated Fe concentration recorded for this study. This result is similar to that recorded by Giarratano *et al.* (2010) who noted that Fe concentrations from their

study must have been from natural origins as there would not have been a human activity that could have caused such a metal input into the system.

According to Hennig (1985), the Fe concentrations in coastal waters at sites along the west coast of the Cape Peninsula were 15 µg/L at Green Point and Milnerton during winter with lower values being recorded during spring (<4.5 µg/L) of 1979. Quick and Roberts (1992) reported Fe surface water values of 22.8 µg/L at Milnerton and 6.78 µg/L at Green Point. During this study, the Fe concentration in surface coastal waters were very low (mean = 1.7 µg/L) as the target set for South Africa is 25 µg/L (DWAF, 1995). Only one elevated mean Fe concentration was recorded at Bloubergstrand during winter 2010 (± 34 µg/L). The results of the present study therefore correspond to elevated Fe concentrations being recorded in winter 2010. The elevated Fe concentrations at Bloubergstrand could be as a result of currents carrying Fe from Milnerton towards Bloubergstrand (Quick & Roberts, 1992). The presence of the refinery and household chemical industries along the Diep River could be a contributing factor to elevated Fe concentrations in the coastal waters near its mouth.

The effect of industrial sources of metals into the coastal environment is evident in the sediment concentrations. Hennig (1985) reported the following dry weight Fe metal concentrations in sediments: Hout Bay: 510 µg/g; Green Point: 1904 µg/g; Milnerton: 519 µg/g and Bloubergstrand: 268 µg/g. During the present study, the following mean sediment Fe concentrations were recorded: Hout Bay: 665.9 µg/g; Green Point: 2409.75 µg/g; Milnerton: 473.9 µg/g; Bloubergstrand 444.2 µg/g. These Fe sediment concentrations are higher than the concentrations recorded by Hennig (1985) for Hout Bay, Green Point and Bloubergstrand. Green Point, in particular, had very high levels of Fe (2409.75 µg/g) which was 23% higher than that recorded previously (Hennig, 1985) for the same site.

Manganese is an essential micronutrient for plants and animals (Duffus, 1980). It is a functional component of nitrate assimilation and an essential catalyst of numerous enzyme systems in animals, plants and bacteria (Duffus, 1980; Hutzinger 1980). Manganese is the eighth most abundant metal in nature, and occurs in a number of ores. In aquatic ecosystems, manganese does not occur naturally as a metal but is found in various salts and minerals, frequently in association with Fe compounds

(Duffus, 1980). Sources of Mn in the natural environment include soils, sediments and metamorphic and sedimentary rocks. Industrial discharges also account for elevated concentrations of manganese in receiving waters (Duffus, 1980; Hutzinger 1980). According to Hennig (1985) the maximum surface water Mn concentration in Table Bay was 1.8 µg/L. The highest concentrations were found at Milnerton and Bloubergstrand. These results were supported by the present study where elevated (>0.1 µg/L) Mn concentrations in surface water were reported at Milnerton (0.1 µg/L) and Bloubergstrand (1.0 µg/L).

According to the WHO (1982) the anthropogenic sources of Mn include: the steel industry, in the manufacturing of dry cell batteries; the fertilizer industry (manganese is used as a micro-nutrient fertilizer additive) and the chemical industry in paints, dyes, glass, ceramics, matches and fireworks. There are no mining activities in the study area and hence the main source of Mn could be from the chemical activities within the industrial areas. The elevated levels in winter could be as result of stormwater runoff. Hennig (1985) reported Mn dry weight concentrations in sediments in 1980 at the various sites as follows: Hout Bay: 8.0 µg/g; Green Point: 21.5 µg/g; Milnerton: 8.0 µg/g and Bloubergstrand: 12.0 µg/g. The sediment Mn recorded in the present study was similar to those reported by Hennig (1985).

Copper occurs naturally in the environment and its concentrations range between 0.03 and 0.38 µg/L (average of 0.25 µg/L) with concentrations as high as 2 µg/L occurring in the marine environment (Riley & Chester, 1983). The distribution of Cu is not uniform in the oceans, with lower concentrations occurring at the surface than at depths (Riley & Chester, 1983). According to Hennig (1985) the average Cu concentrations in South African waters in 1985 was 0.899 µg/L. The mean Cu concentration recorded during the present study was 0.002 µg/L, much lower than that recorded in the 1980's. According to Riley & Skirrow (1975) seawater can retain a maximum of 50 µg/L of Cu and the levels of concentrations are influenced by riverine sources or shelf sediments.

The anthropogenic sources of copper include: metal plating operations; jewellery and ornamental industries; electrical wiring industries; electronic industries and antifouling paints (WHO, 1982). From this study higher Cu concentration were recorded in winter 2010 (Figure 3-11) and this could be indicative of freshwater sources of Cu, in

particular, at Hout Bay and Green Point. There are, however, no major industrial activities involving Cu along the west coast of the Cape Peninsula. However, Hout Bay is close to a harbour with shipyards (Abdallah & Abdallah, 2008) and this could have contributed to the higher levels of Cu at that site. This suggestion is supported by the elevated concentrations of Cu in the sediment at Hout Bay (Figure 3-3). Hennig (1985) reported on sediment Cu concentrations at various sites in South Africa, including sites similar to sampling sites of the present study. Copper dry weight concentrations recorded by Hennig (1985) at the various sites were: Hout Bay 4.77 µg/g; Green Point: 4.5 µg/g; Milnerton: 10 µg/g; Bloubergstrand: 0.3 µg/g, with a mean concentration of 3.994 µg/g dry weight for all sites combined. Mean Cu concentration reported in this study was 2.205 µg/g dry weight, a 55% decrease since the 1980's.

Zinc occurs abundantly in the natural environment and is comparatively speaking, non-toxic in low concentrations, and therefore Zn in the marine environment is considered to pose no health risk to humans when exposed to low concentrations (Hutzinger, 1980; Riley & Chester, 1983). According to Riley & Chester (1976), the average concentration of Zn in marine water is 5 µg/L. Concentrations, however, range between 0.003 and 0.59 µg/L with an average of 0.39 µg/L being reported for most Zn species in the natural environment (Riley & Chester, 1983). The average Zn concentration in South African surface marine waters has been reported to be 6.59 µg/L (Hennig, 1985). The mean Zn concentrations in surface waters recorded from the present study was 0.11 µg/L, well below the average reported for South Africa by Hennig (1985). There was only one peak in surface water Zn concentrations during this study in winter 2010 at Milnerton (2.5 µg/L). This elevated level of Zn is also higher than that reported by Quick and Roberts (1992) where 0.01 µg/L was recorded. According to Riley & Skirrow (1975), the toxicity of Zn increases in the presence of Cd. From this study, there was an elevated level of Cd at Milnerton during winter 2010, the same as Zn (Figure 3-12 and Figure 3-13).

Anthropogenic sources of Zn include: waste from brass and Zn metal works, Zn and brass plating, steel galvanisation and stainless steel tableware manufacturing; waste from viscose rayon yarn and fibre production; waste from battery production; waste from paint and dye manufacturing; anti-corrosion in cooling towers; waste from pulp

and paper manufacturing (WHO, 1982). Some of these industrial activities are prevalent in the Paarden Island industrial area and therefore the probable source of elevated Zn waters concentrations at Milnerton. Given that the elevated Zn values were recorded in winter (the rainfall season), the Zn in waste from industrial activities from the surrounding area could have been the cause for the higher Zn values recorded at Bloubergstrand.

The effect of metal-laden effluent from Milnerton on Bloubergstrand is evident in sediment Zn levels of the present study. Although elevated levels of Zn were recorded at Milnerton in the surface waters during winter 2010, there were elevated levels of Zn in the sediment at Bloubergstrand. According to Quick and Roberts (1992), there are two current systems in Table Bay, one in the central bay waters, and the other a bimodal longshore current system. Depending on the wind direction, the bay either circulates in either a clockwise or anti-clockwise direction. Measurement in the bay indicated that the anti-clockwise (northerly) current has a surface flow between 0.2 and 0.3 ms^{-1} (maximum 0.6 ms^{-1}) and is driven by southerly winds. The clockwise (southerly) current was approximately 0.1 ms^{-1}. The predominant surface current was in a northerly direction. According to Van Ieperen (1971), nearshore currents are dominated by swell direction which for 70% of the time comes form the southwesterly sector (200° - 260°). The remainder comes form the west-southwest (15% of the time), west (10% of the time) and from the northwest (5% of the time). Because the incidental swell comes mainly from the southwest and strikes the shore obliquely, the dominant longshore current is in a northerly direction (Van Ieperen, 1971). The northward movement of current results in a northward movement of metals released at Milnerton and could be the cause of elevated metal concentrations (in particular, Zn) at Bloubergstrand given the topography and bathymetry (Shannon, 1985) of the area at Bloubergstrand.

The source of Cd though is difficult to ascertain, whether from natural or anthropogenic sources. Goldberg *et al.* (1983) proposed that Cd taken up by mussels could also be related to upwelling. Although Cd stems from land stormwater runoff, higher Cd values recorded during spring 2010 and summer 2010 (Fig 4-5) could be elevated due to upwelling events. This argument is not proposed for Hout Bay and Milnerton as the lower Cd concentrations in spring 2010 as these areas are not

directly linked to upwelling centres. The bays are semi-enclosed areas and therefore could support the proposal that sources Cd be from upwelling events. Scarborough, Green Point and Bloubergstrand on the other hand are more exposed to open ocean (natural) sources of elements such as upwelling events. However, the real source of Cd in the region requires further investigation.

Cadmium occurs in the environment as a result of natural weathering and erosion in rivers that are transported to coastal waters where the average concentration in seawater is 0.1 µg/L or less (WHO, 1992). The average concentration of Cd in South African surface marine waters has been reported as 0.108 µg/L (Hennig, 1985). The mean Cd concentration reported in the present study was 0.004 µg/L, well below the average for coastal waters recommended by the WHO (1992). However, there was one peak in Cd levels in seawater recorded at Milnerton during winter 2010. A probable reason could have been increased Cd levels in river runoff due higher rainfall during winter. The vertical distribution of Cd in ocean waters is characterised by surface depletion and deep water enrichment (DWAF, 1995). These authors further noted that this distribution in the environment could be the result of Cd uptake by phytoplankton in surface waters and its transportation to depths could be the result of Cd uptake by phytoplankton. Furthermore, Cd is enriched in surface waters of upwelling areas (not linked to pollution sources) which also leads to elevated levels of Cd in plankton (WHO, 1992).

Cadmium levels between 30 and 1000 µg/g have been reported for marine sediments (WHO 1992). Hennig (1985) reported Cd values <0.1 µg/g dry weight between Hout Bay and Bloubergstrand. During the present investigation the mean Cd concentration in sediments was 0.29 µg/g dry weight. However, Cd values >0.4 µg/g were recorded during winter 2010 at Bloubergstrand. According to WHO (1992), anthropogenic sources of Cd include: waste from manufacturing protective plating for steel; waste from manufacturing stabilisers for PVC; waste from manufacturing plastics and glass; electrode material in nickel-cadmium batteries; and, waste from manufacturing various alloys. Of the various sources of Cd entering the environment, at the global level, the smeltering of non-ferrous metal ores has been identified as the largest source of Cd release into the environment (WHO, 1992). Although the values displayed high variability, the relatively high Cd sediment values at Bloubergstrand

could have arisen from runoff in the adjacent area as Bloubergstrand has been an area where housing developments has taken place over the past 10 years. However, the specific source of elevated Cd in the areas adjacent to Bloubergstrand is difficult to ascertain.

According to the WHO (1992), anthropogenic sources of Pb include: waste from manufacturing of car batteries, metal plating, and petroleum additives; waste from printing, pigment, fuel, photographic, match and explosive industries; waste from paint and pigment industries. The Caltex refinery as well as other industrial activities within the area at Milnerton (*e.g.* Paarden Island) could contribute to anthropogenic sources of Pb and it is likely that Pb from these sources have resulted in elevated Pb concentration at Bloubergstrand.

Lead is not considered to be an essential element (Hutzinger, 1980) but does occur in the marine environment due to natural sources from weathering of rocks, volcanism and forest fires (Riley & Chester, 1983). The concentration of dissolved Pb in ocean waters ranges from 0.5 to 3 µg/L (Riley & Skirrow, 1975). The average lead concentration in South African surface marine waters in the 1980's ranged between 0.025 and 0.15 µg/L (Hennig, 1985). Compared to these values, Pb concentrations were higher (1.5 2 µg/L) at Green Point, Milnerton and Bloubergstrand during spring 2010 and increased at Green Point during winter 2010. The mean Pb concentrations for all sites recorded in the present study was 0.003 µg/L. Pb concentrations in excess 0.1 µg/L were recorded during winter 2010 at Milnerton and Bloubergstrand in the present study. This result reported here is higher than that reported by Hennig (1985). A possible contributing factor to the elevated reading is that the Diep River opens adjacent to Milnerton and the current close inshore could move that inshore waters to Bloubergstrand via the counter current system. Within the Diep River system is an oil refinery and the emissions from this plant as well as other industrial activities is thought to enter the ocean via the Diep River catchment system (Quick & Roberts, 1992). Shuping (2008) reported 2.09 µ/L Pb in marine water at the mouth of the Diep River which is indicative of elevated Pb concentrations when compared to the average Pb concentrations for SA waters (between 0.025 and 0.15 µg/L) reported by Hennig (1985).

The anthropogenic sources of Pb noted previously are prevalent at Hout Bay and Milnerton (*e.g.* fuel wastes at Hout Bay and petrol additives, fuel wastes, paint and pigment wastes at Milnerton). The previous levels of sediment Pb (as dry weight) along the west coast of the Cape Peninsula were reported by Hennig (1985) as follows: Hout Bay: 1.5 µg/g; Green Point: 9.3 µg/g; Milnerton: 1.3 µg/g and Bloubergstrand: 0.4 µg/g. The results of the present study indicate that Pb levels in sediments have increase since then. Mean Pb levels per site were Hout Bay: 1.7 µg/g; Green Point: 3.67 µg/g; Milnerton: 2.1 µg/g and Bloubergstrand: 1.32 µg/g. Only Green Point has lower levels of Pb than that reported by Hennig (1985). The result therefore suggests that increased industrial activities have resulted in increased levels of Pb in the sediment at Hout Bay, Milnerton and Bloubergstrand.

When comparing the metal concentrations within the sediment and surface waters along the west coast of the Cape Peninsula to that of previous values (Hennig, 1985), it is apparent that the metal concentrations have not increased as would have been expected given the rapid rate of industrial development and urbanisation in the Western Cape. The lack of an increased anthropogenic input of metals into the environment is evident in the low contamination factor values recorded for the study period, as evident in Table 3-4. Only Zn at Bloubergstrand, Cd at Bloubergstrand and Cu at Green Point showed dramatic increases from that reported by Hennig (1985) values (CF > 50). The contamination factor (CF) is a means of quantifying the magnitude of pollution by the various metals (El-Sammak & Aboul-Kassim, 1999). The results of this study suggests that pollution is highest during winter 2010 (CF = 20.83), a three-fold increase from other seasons.

The uptake of metals by marine fauna and flora is an important factor to consider as a possible reason for the metal concentrations reported in the present study. Metal bioavailability in coastal wasters is dependent on seasonal patterns and the extent of annual variations of accumulated metals (Przytarska *et al*., 2010). Although metal concentrations in the sediment were lower than that reported by Hennig (1985), these metals could have been taken up by marine organisms. According to Mubiana *et al.* (2005), differences between metal concentrations at various sites could result from different physiological handling of the metals taken up. According to these authors, summer-autumn is the post-spawning period when mussels recover after energy-

demanding spawning and gather energy reserves. As such, they are highly dependent on external environmental conditions, including the availability of metals. The level of metals in the coastal biota is important to measure as it indicates whether metals bioaccumulate in organisms and consequently could pose threats to the health of the organisms.

Since the Western Cape has a Mediterranean type climate (Shannon, 1985) with winter rainfall, the runoff from freshwater (and hence landward side) is considered to be the major contributor to the increase pollution of metals in the area. This trend is also particularly evident in the pollution load index (PLI) for the study area (Table 3-5). Pollution load was lowest at Scarborough and highest at Hout Bay, although the difference between Hout Bay, Green Point and Bloubergstrand was less than 0.1. Hout Bay is a commercial fishing harbour that is a popular tourist destination. The area has seen a rapid rate of informal settlement developments which in turn has led to increase sewage discharge into the bay. A study by Fricke *et al.* (1979) recorded an average of 510.4 ± 43.53 µg/g dry weight Fe in surface sediment concentrations sampled during summer 2010. The mean Fe sampled at Hout Bay for this study was 665.2 ± 883.15 µg/g dry weight, which is higher than previous 1980 levels.

Hout Bay is an area that has developed over the last 15 years in terms of residential and commercial use (River Health Programme, 2005). In addition to the shipyard activities as fish factory effluent discharge into the bay, human activities are influencing the health of the bay. According to the River Health Programme (2005), a number of important factors influence the area. The Longkloof weir, a concrete structure, canalises the river for a short stretch and restricts the natural function of the river flowing into the bay. Reeds have proliferated in the lower reaches of catchment area due to elevated nutrient loads and reduction of flow. These clog the channel, hold back flood waters and require continual maintenance. Small dams and abstractions for livestock watering and garden irrigation have drastically reduced summer flow in the lower reaches of the catchment area. Runoff, storm water discharges and seepage from septic tanks reduce the water quality in the lower reaches. Low flow and poor water quality have resulted in the disappearance of pollution-sensitive invertebrates.

Cultivation of farm produce has resulted in the removal of reeds along the river banks, increasing erosion and causing deposition of large amounts of sediments into the estuary and ultimately the bay. The coastal activities have influenced the quality of the water in the bay and it is argued here to be a significant contributor to the deterioration of the quality of the water and sediment in Hout Bay. This relationship needs to be further investigated and conducting correlation analyses on environmental conditions with that of the quality of the marine environment. This however might prove difficult, given the poor correlations between variables such as seasonal relationships water and sediment (see Table 3-3). As evident from Table 3-3, a spatial study is better suited for correlation analysis.

3.6. Conclusion

The result of the present study has provided information about the levels of metals in coastal waters and sediments of the western coastline of the Cape Peninsula, Cape Town. Although levels of metals have increased over the past 30 years, the levels are low compared to other industrialised coastal cities in Europe (WHO, 1992). The CF analysis suggests that Scarborough was the least affected by pollution of the 5 sites studied whereas Bloubergstrand was the most affected by pollution, in particular, as a result of Zn concentrations in the sediment. The CF values hence suggests that the increase in metals in the area has resulted in metal accumulation in the sediment as there are not enough studies in this part of coast to be able to compare the sediment quality. Information collected through the present study can be used as baseline data for future monitoring of metal pollution in the Western Cape.

Table 3-1. Coordinates of the five sites along the west coast of the Cape Peninsula. * indicates control site.

Site	S	E
Scarborough*	34° 11’ 58.98”	18° 22’ 17.96”
Hout Bay	34° 02’ 53.55”	18° 21’ 38.43”
Green Point	33° 54’ 19.84”	18° 23’ 50.80”
Milnerton	33° 53’ 41.07”	18° 28’ 48.00”
Bloubergstrand	33° 48’ 11.83”	18° 27’ 38.55”

Table 3-2. Temporal (seasonal) and spatial Pearson's product moment correlations between metal concentrations in the sediment (µg/g dry weight) and water (µg/L) sampled seasonally from autumn 2010 to autumn 2011. Significant correlations ($p < 0.05$) are highlighted in bold.

		Fe	Mn	Cu	Zn	Cd	Pb
Metal sea water vs sediment correlations (for all data combined)		-0.1005	-0.1193	-0.0538	0.0018	0.0042	-0.0185
Season	**Autumn 2010**	0.2079	-0.0577	0.1922	0.0315	**0.9821**	-0.0207
	Winter 2010	-0.2455	-0.2271	-0.0450	-0.1280	-0.1619	-0.1142
	Spring 2010	-0.0778	0.0795	-0.0641	-0.0422	-0.0441	-0.1346
	Summer 2010	-0.0711	0.1757	-0.1067	**0.4869**	-0.1057	**0.3847**
	Autumn 2011	0.1427	0.0962	-0.0074	**0.3738**	**0.4290**	-0.1624
Site	**Scarborough**	-0.0155	-0.0195	0.0220	-0.0533	-0.1002	**-0.3888**
	Hout Bay	**0.3920**	**0.3363**	-0.1186	0.0971	0.1514	**0.4167**
	Green Point	**0.4289**	0.2014	-0.1284	-0.0800	**0.8618**	-0.2353
	Milnerton	-0.0060	-0.2007	-0.0909	0.1313	0.0209	-0.0239
	Bloubergstrand	**-0.3525**	0.1954	**0.4377**	-0.0272	**0.5209**	0.0864

Table 3-3. Background sediment metal concentrations (µg/g) of the different sites as recorded by Hennig (1985). Data in parenthesis from Kiviets, (unpubl. data).

	Year	Mn	Fe	Cu	Zn	Cd	Pb
Scarborough	1986	(4.24)	(122)	4.77	135	3.77	0.86
Hout Bay	1977	8.0	510	0.4	2.8	0.18	1.5
Green Point	1980	21.5	1904	4.5	11.8	0.1	9.3
Milnerton	1980	8.0	519	10	10	(1.83)	1.3
Bloubergstrand	1981	12.0	268	0.3	0.41	0.05	0.4

Table 3-4. Sediment contamination factors (CF) of metals at different sites from autumn 2010 to autumn 2011 based on values from Hennig (1985).

	Mn	Fe	Cu	Zn	Cd	Pb	Average
Autumn 2010							
Scarborough	0.0001	0.0001	0.0000	0.0000	0.0000	0.0001	0.0000
Hout Bay	1.2723	1.4064	1.7724	6.1513	0.5696	2.5202	2.2820
Green Point	0.3973	0.0104	0.1582	0.0739	19.6324	0.2181	3.4150
Milnerton	0.8136	0.8461	0.0361	0.2308	0.0304	1.3447	0.5503
Bloubergstrand	0.9442	2.8358	5.0447	16.7051	0.9169	3.6469	5.0156
Average	0.6855	1.0198	1.4023	4.6322	4.2299	1.5460	2.2526
Winter 2010							
Scarborough	0.3668	0.2169	0.0000	0.0000	0.0078	0.1992	0.1318
Hout Bay	1.2411	1.4373	0.0000	0.0000	0.5512	0.7174	0.6578
Green Point	0.9900	1.3519	0.0000	0.2108	0.3948	0.4838	0.5719
Milnerton	0.6962	1.0401	0.1034	1.1178	0.0448	1.7055	0.7846
Bloubergstrand	0.1189	0.5985	0.0000	484.6531	91.5349	3.6330	96.7564
Average	0.6826	0.9289	0.0207	97.1964	18.5067	1.3478	19.7805
Spring 2010							
Scarborough	1.0565	0.6111	0.6987	0.0000	0.0029	0.4048	0.4623
Hout Bay	1.1310	1.0987	44.1030	0.8107	0.3833	0.5143	8.0068
Green Point	0.7580	0.7856	1.1858	2.1961	3.1143	0.4403	1.4134
Milnerton	0.6824	0.8106	0.2460	0.0844	0.0208	1.6604	0.5841
Bloubergstrand	0.6287	1.6866	0.7982	0.1903	0.3537	2.6987	1.0594
Average	0.8513	0.9985	9.4063	0.6563	0.7750	1.1437	2.3052
Summer 2010							
Scarborough	0.5518	0.7095	0.0292	0.0281	0.0036	0.3751	0.2829
Hout Bay	0.9129	1.4631	0.5542	0.1413	0.0336	0.4111	0.5860
Green Point	1.2375	2.2823	1.2839	0.5527	0.1298	0.6455	1.0220
Milnerton	0.6890	1.0300	0.0393	1.7076	0.0222	1.4075	0.8159
Bloubergstrand	0.9366	2.3966	1.8839	70.1446	0.0000	3.1387	13.0834
Average	0.8656	1.5763	0.7581	14.5149	0.0378	1.1956	3.1580
Autumn 2011							
Scarborough	1.1535	0.6035	0.2110	0.0000	0.0157	0.7089	0.4488
Hout Bay	1.5288	1.7108	8.0555	0.1307	0.8452	0.8764	2.1912
Green Point	1.1465	1.5841	0.9352	1.0585	0.5044	0.5089	0.9563
Milnerton	1.1667	1.3867	0.3384	0.1859	0.0911	2.3089	0.9129
Bloubergstrand	0.4333	1.2335	3.4497	17.7825	0.8293	1.7615	4.2483
Average	1.0858	1.3037	2.5979	3.8315	0.4572	1.2329	1.7515

Table 3-5. Pollution Load Index (PLI) of metals in sediments at the different sites from autumn 2010 to autumn 2011.

	Autumn 2010	Winter 2010	Spring 2010	Summer 2010	Autumn 2011	Average
Scarborough	0.4385	0.8688	0.8889	0.8918	0.9046	0.7321
Hout Bay	1.0240	0.9946	1.0379	0.9502	1.0439	1.0188
Green Point	0.9122	0.9789	0.9995	0.9862	0.9871	0.9635
Milnerton	0.8979	0.9558	0.9196	0.9504	0.9668	0.9244
Bloubergstrand	1.0613	1.0507	0.9967	1.1127	1.0156	1.0362
Average	0.8668	0.9697	0.9685	0.9782	0.9836	

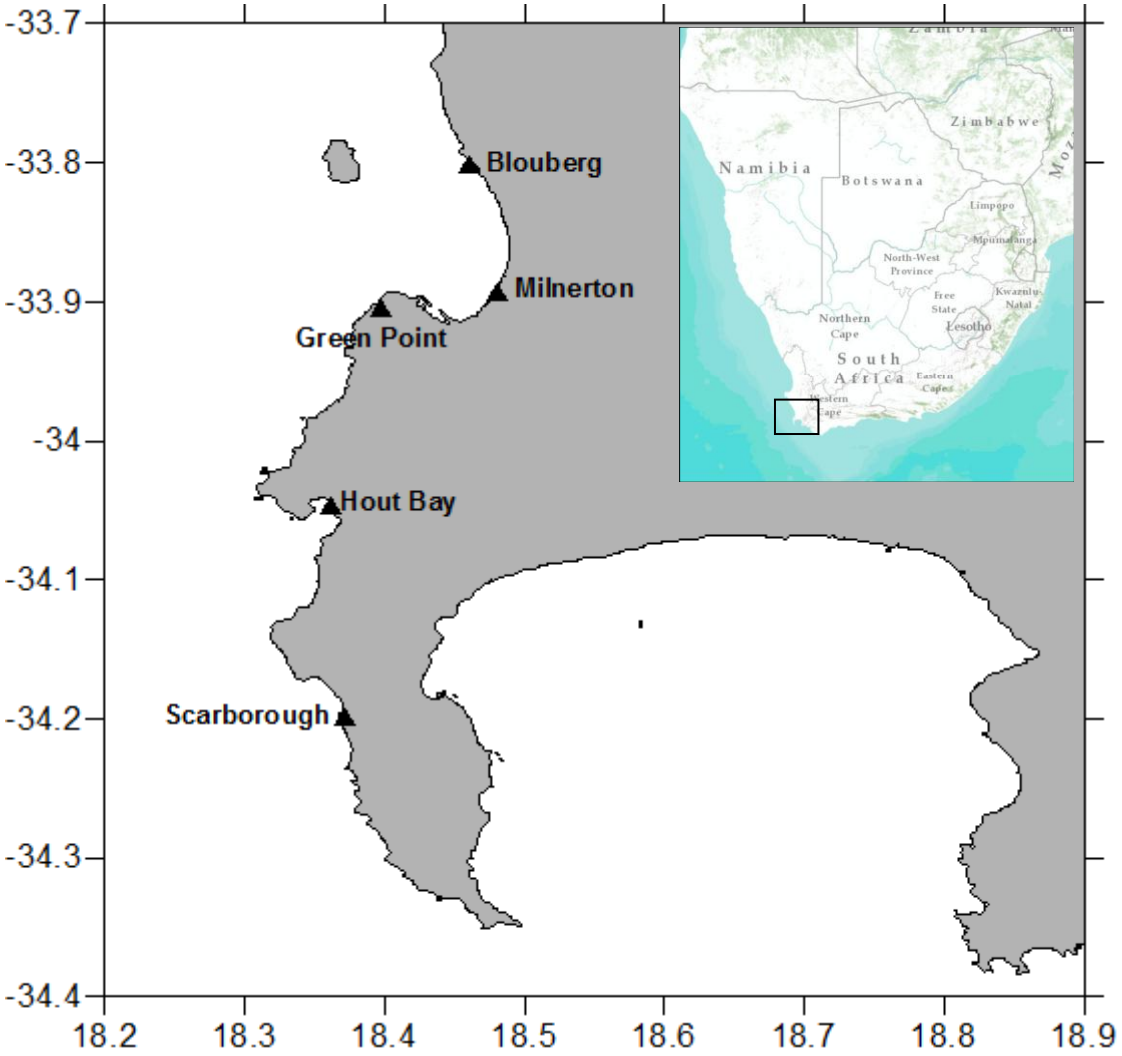

Figure 3-1. Map showing the position of the sampling sites in the Cape Peninsula (from Scarborough to Bloubergstrand).

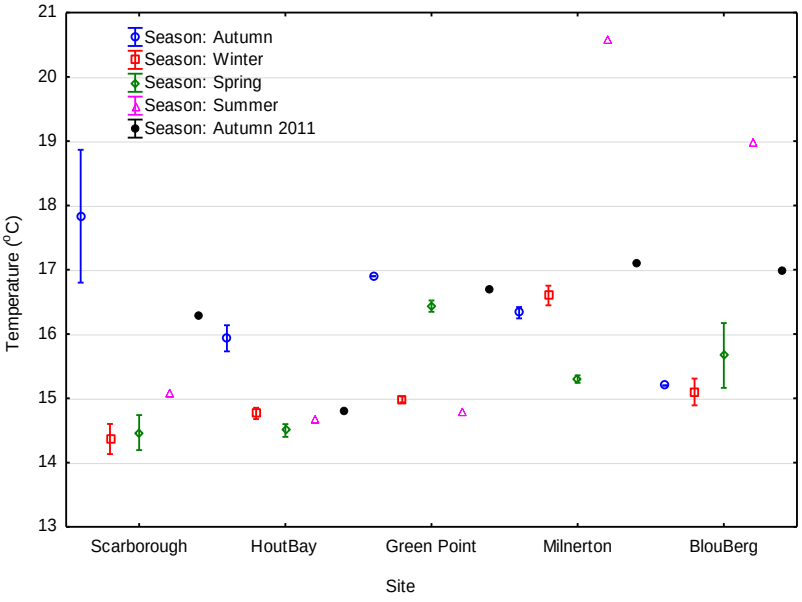

Figure 3-2. Mean water temperature (±SE) measured along the west coast of the Cape Peninsula from autumn 2010 to autumn 2011.

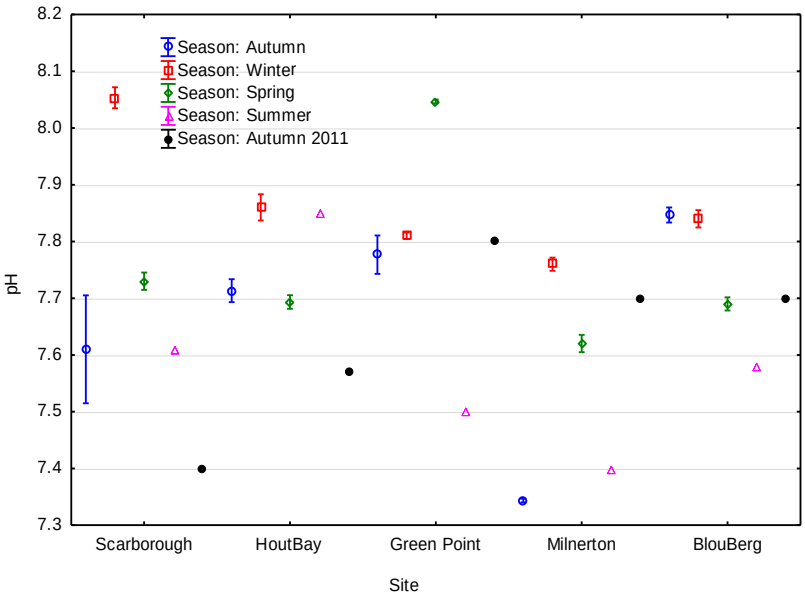

Figure 3-3. Mean pH (±SE) measured along the west coast of the Cape Peninsula from autumn 2010 to autumn 2011.

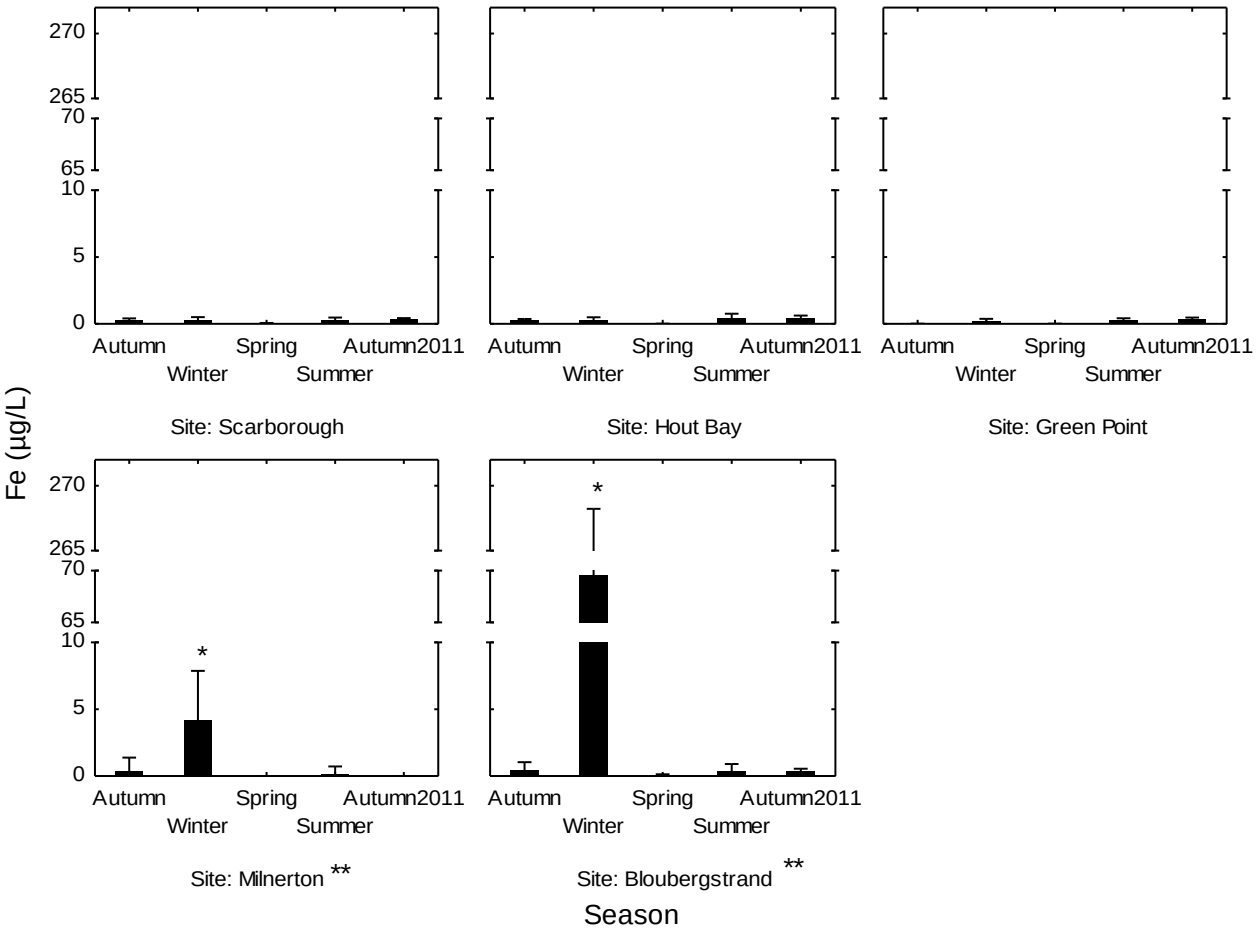

Figure 3-4. Mean Fe concentrations (µg/L) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$). ** indicates significant differences from Scarborough using the Dunnett's post-hoc test ($p<0.05$).

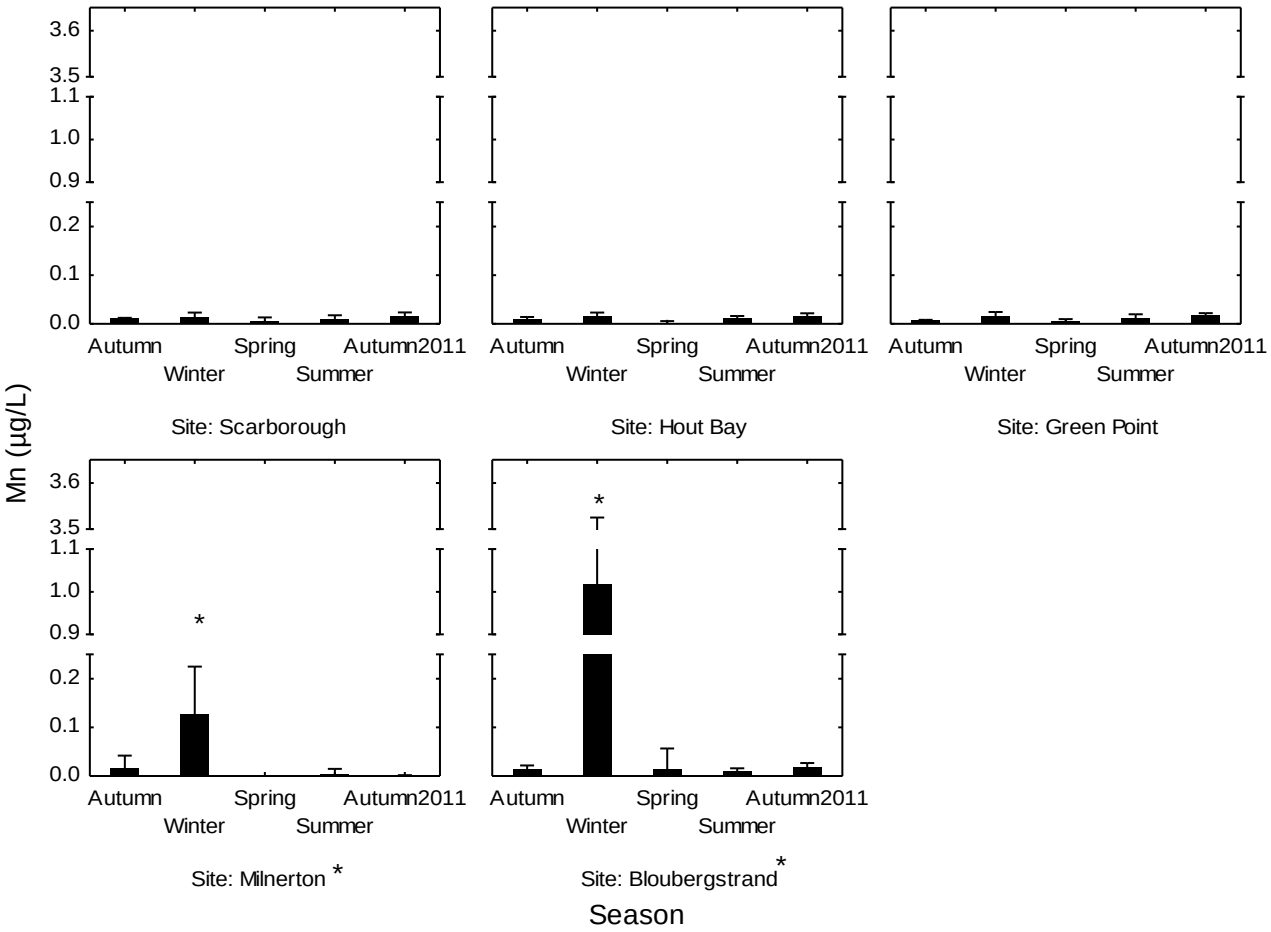

Figure 3-5. Mean Mn concentrations (µg/L) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$). ** indicates significant differences from Scarborough using the Dunnett's post-hoc test ($p<0.05$).

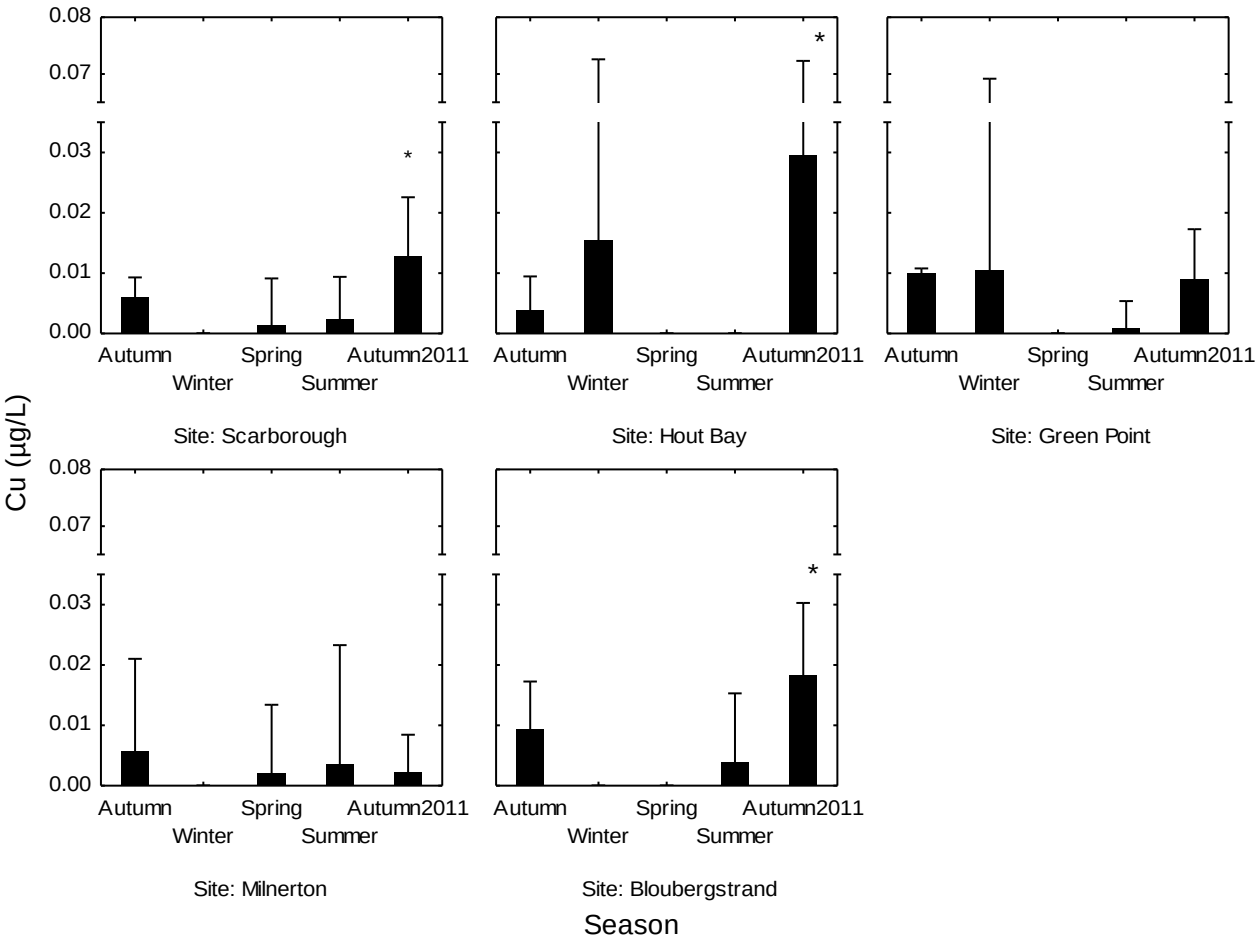

Figure 3-6. Mean Cu concentrations (µg/L) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

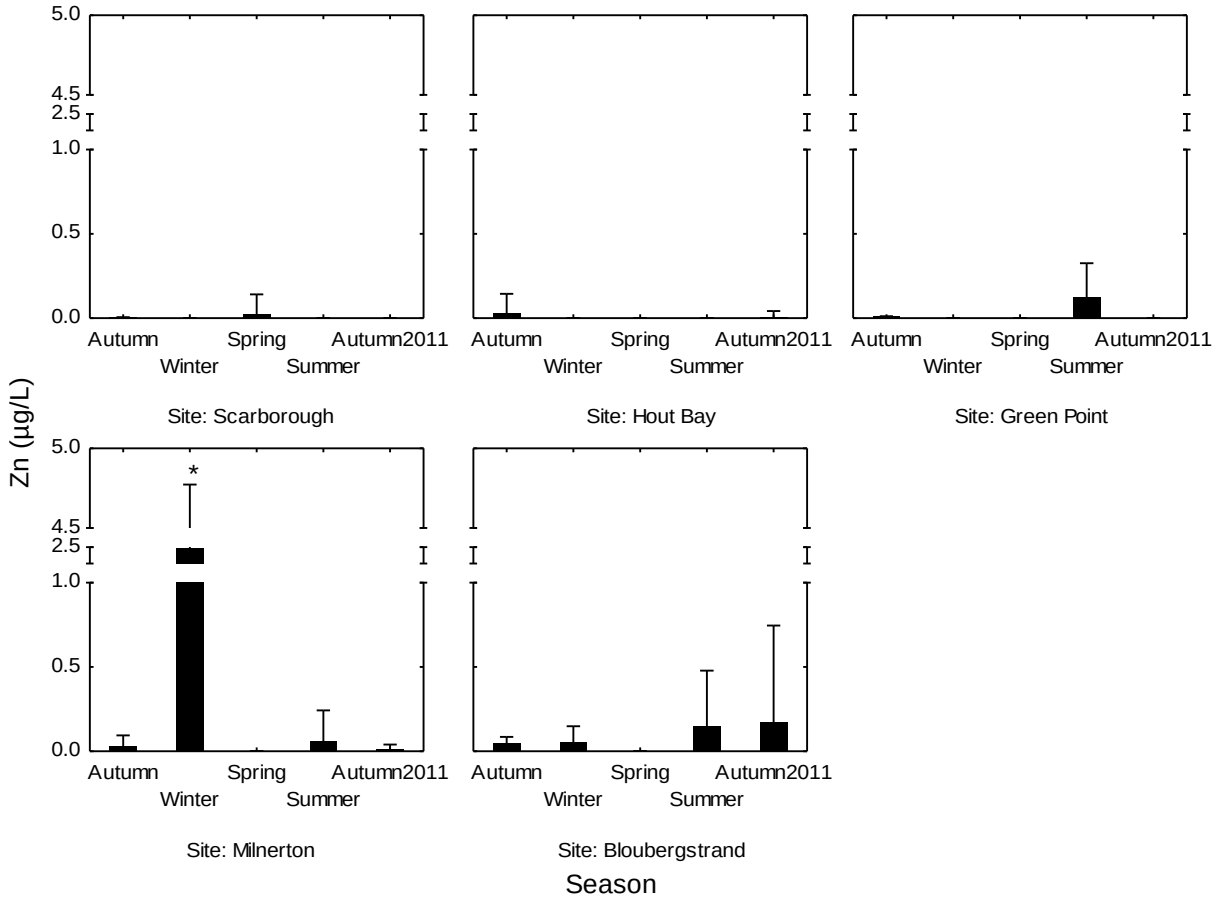

Figure 3-7. Mean Zn concentrations (µg/L) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

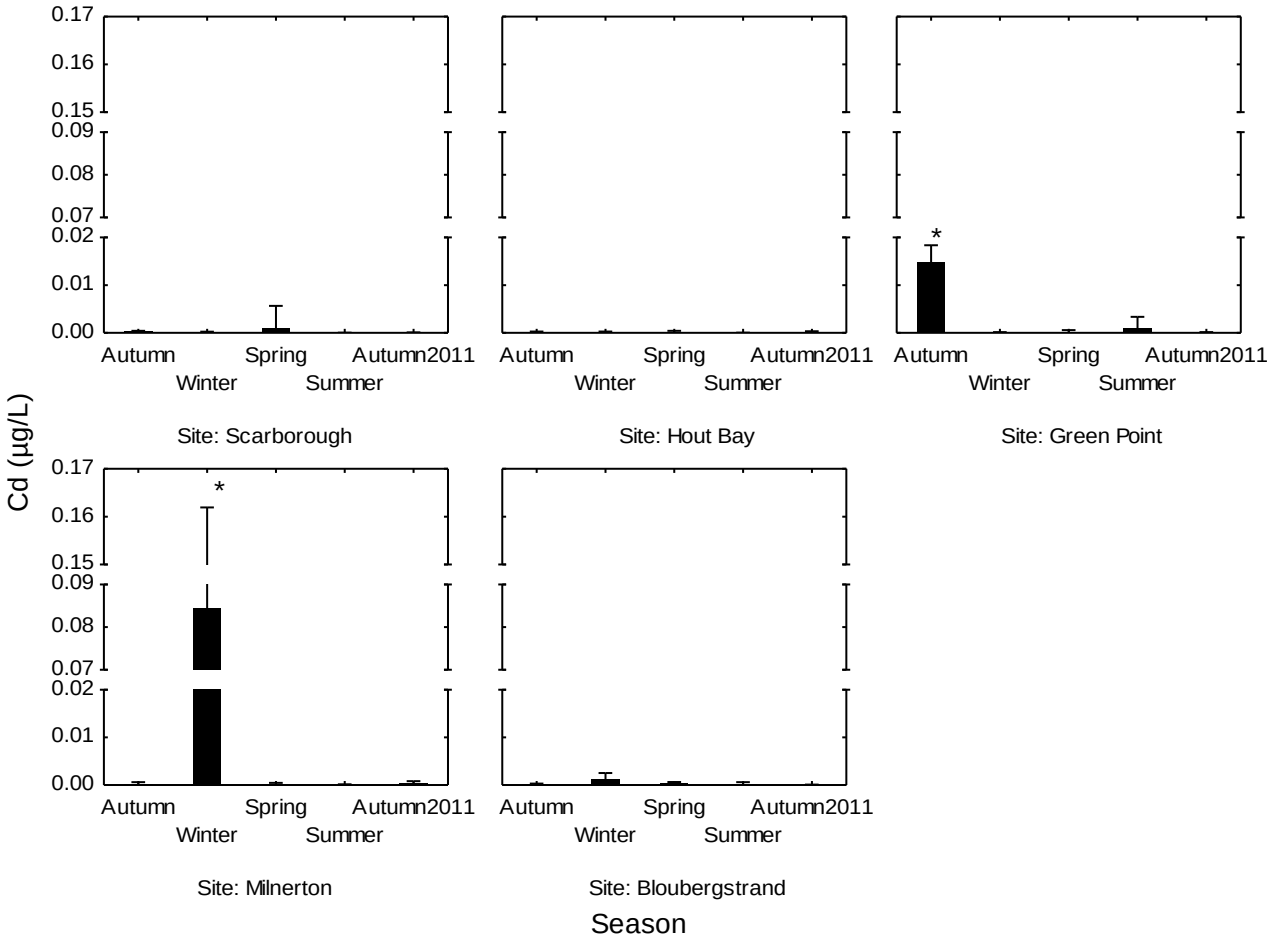

Figure 3-8. Mean Cd concentrations (µg/L) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

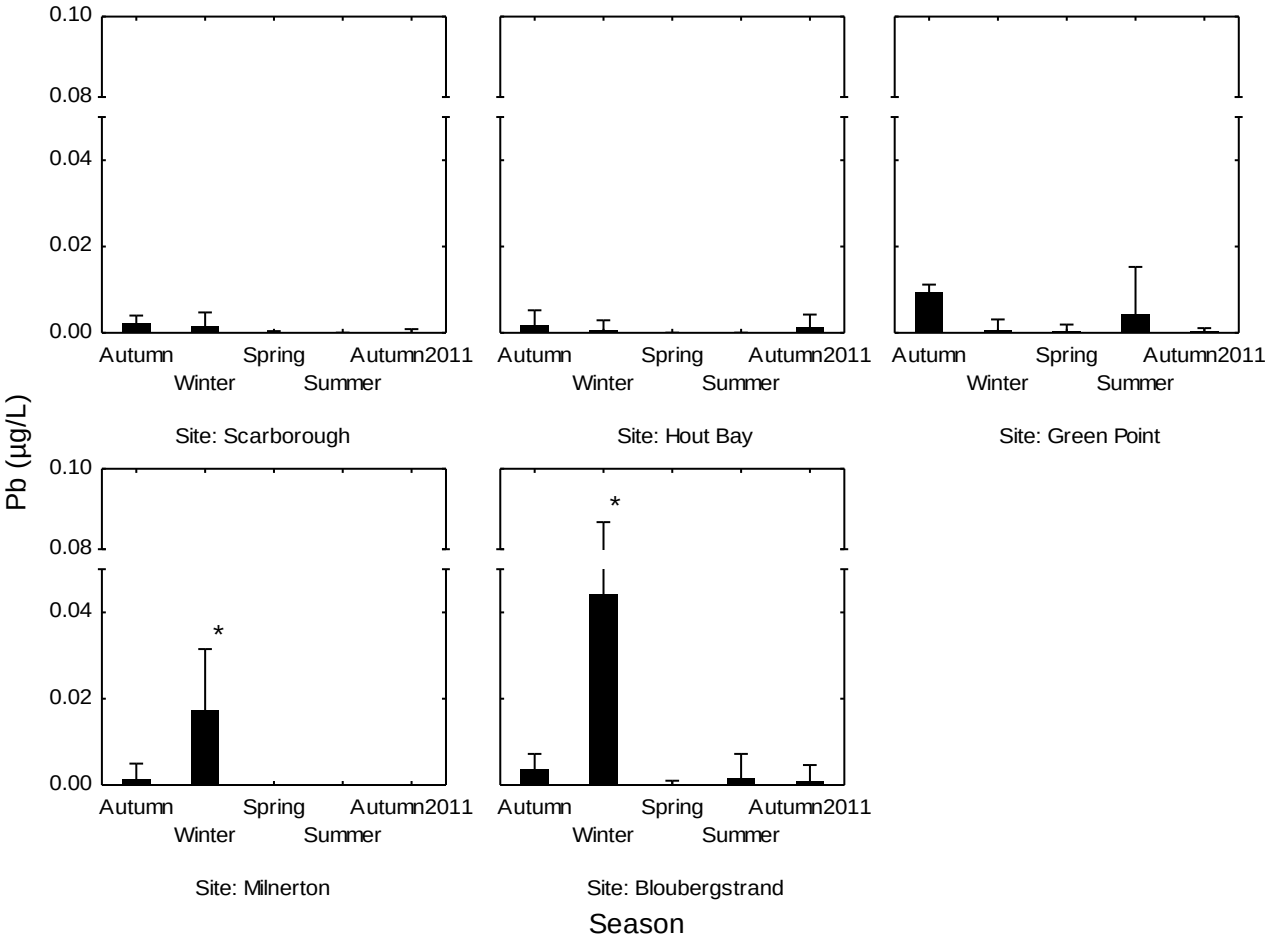

Figure 3-9. Mean Pb concentrations (µg/L) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

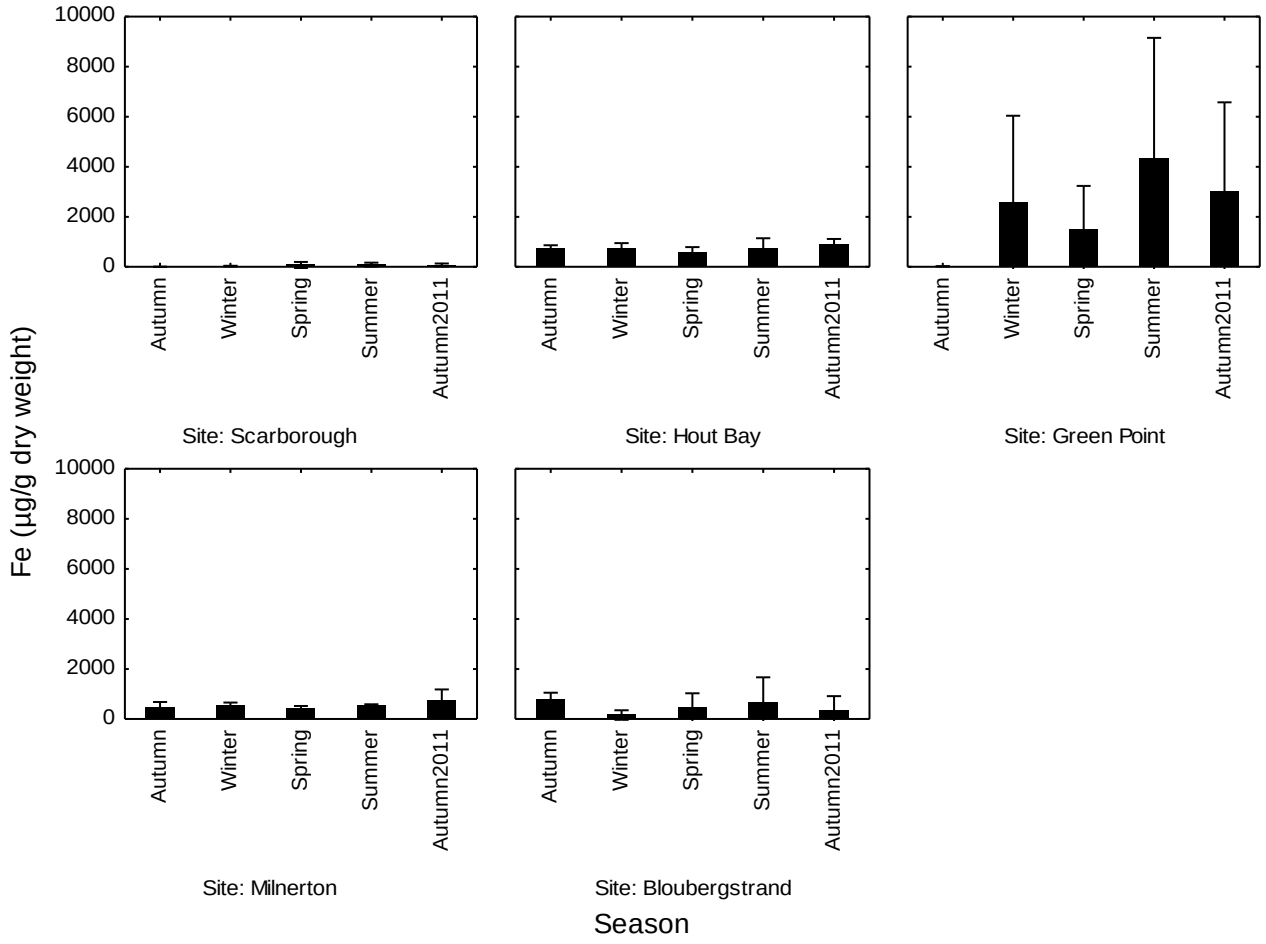

Figure 3-10. Mean Fe concentrations (µg/g dry weight) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

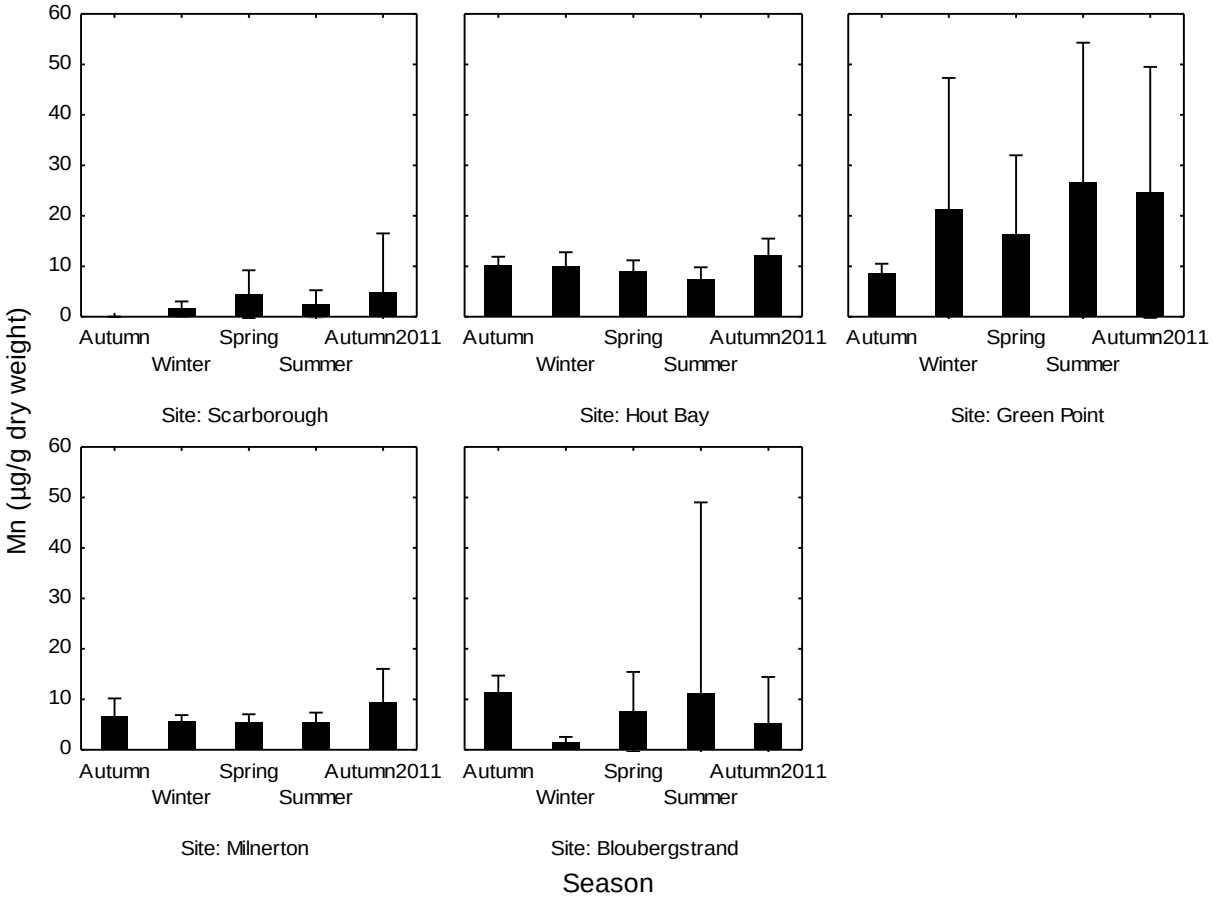

Figure 3-11. Mean Mn concentrations (µg/g dry weight) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

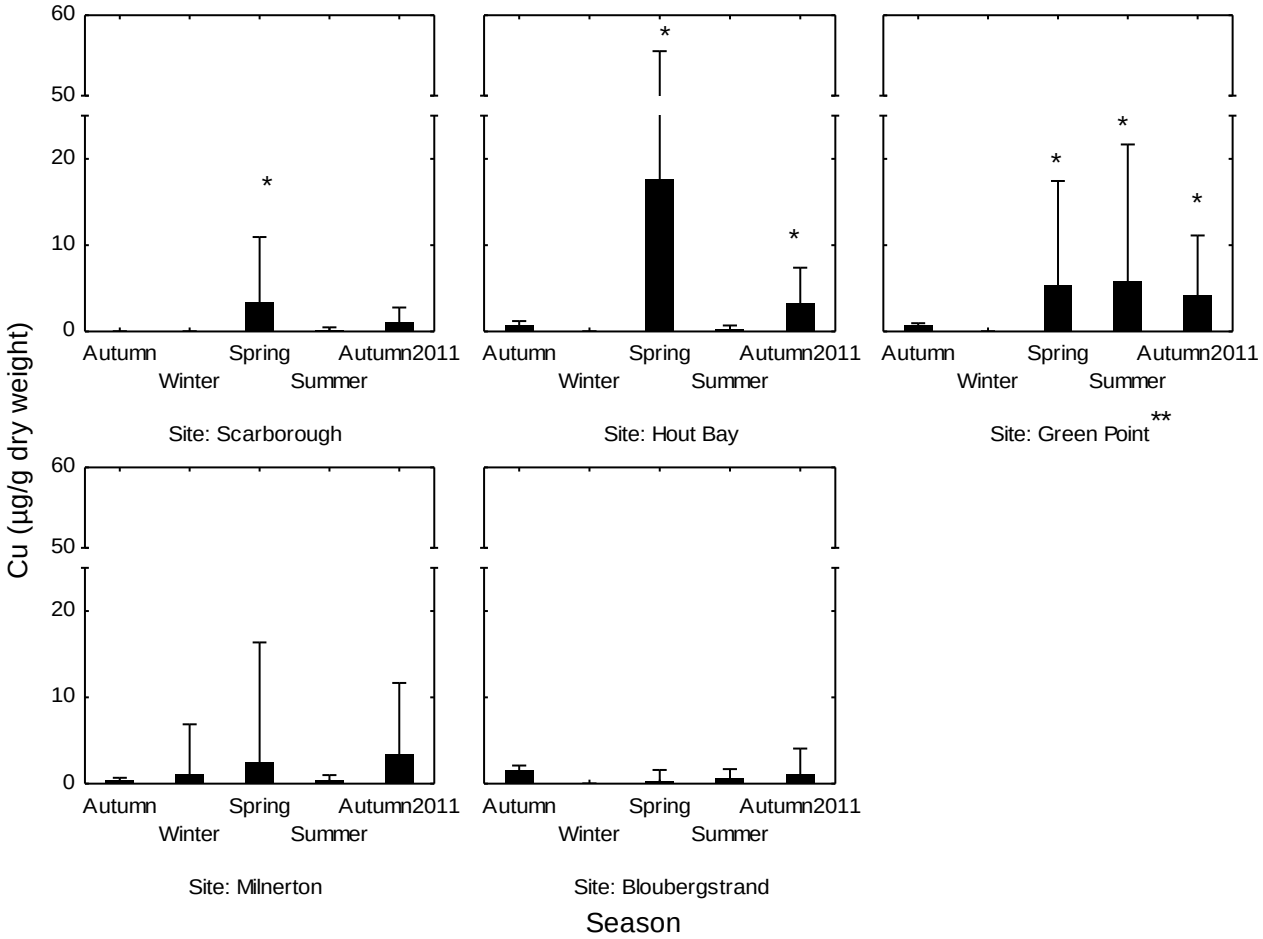

Figure 3-12. Mean Cu concentrations (µg/g dry weight) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$). ** indicates significant differences from Scarborough using the Dunnett's post-hoc test ($p<0.05$).

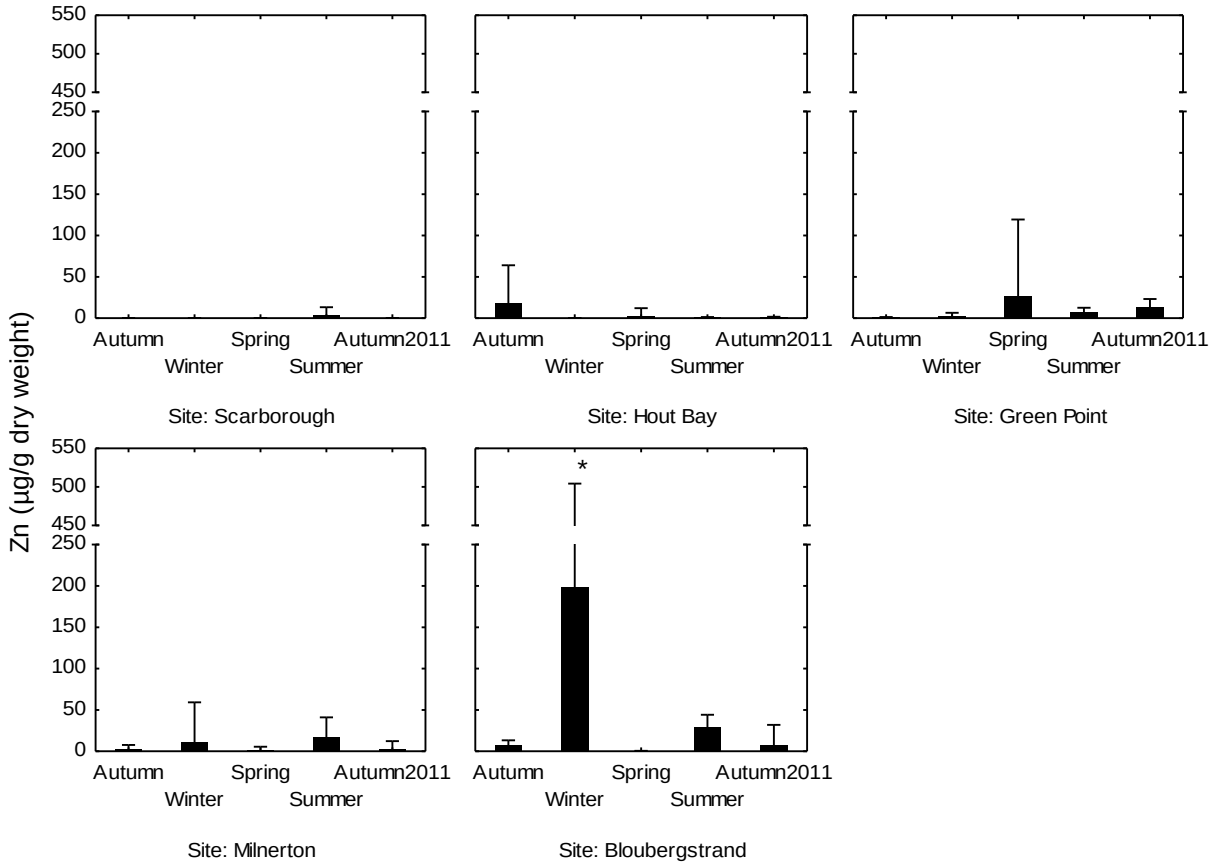

Figure 3-13. Mean Zn concentrations (µg/g dry weight) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

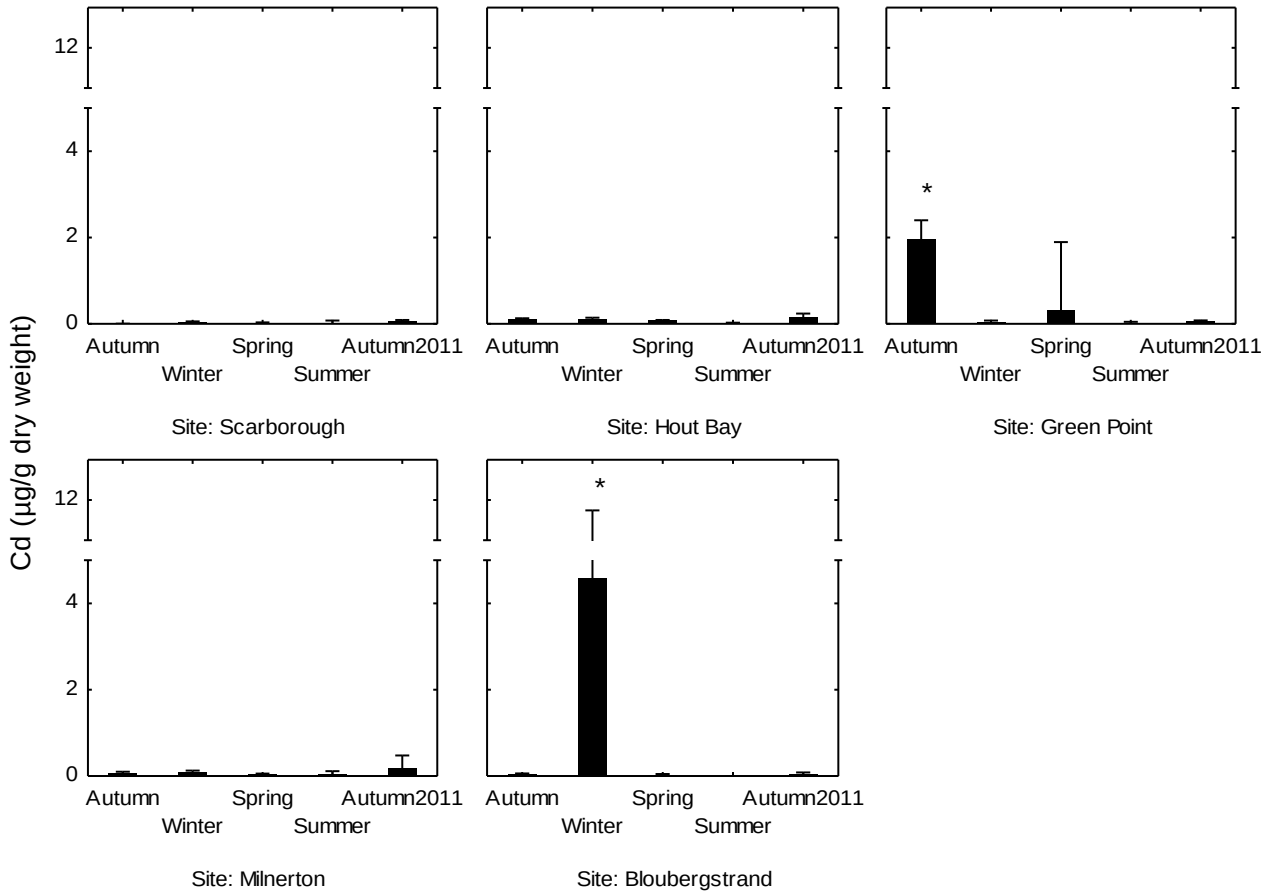

Figure 3-14. Mean Cd concentrations (µg/g dry weight) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$).

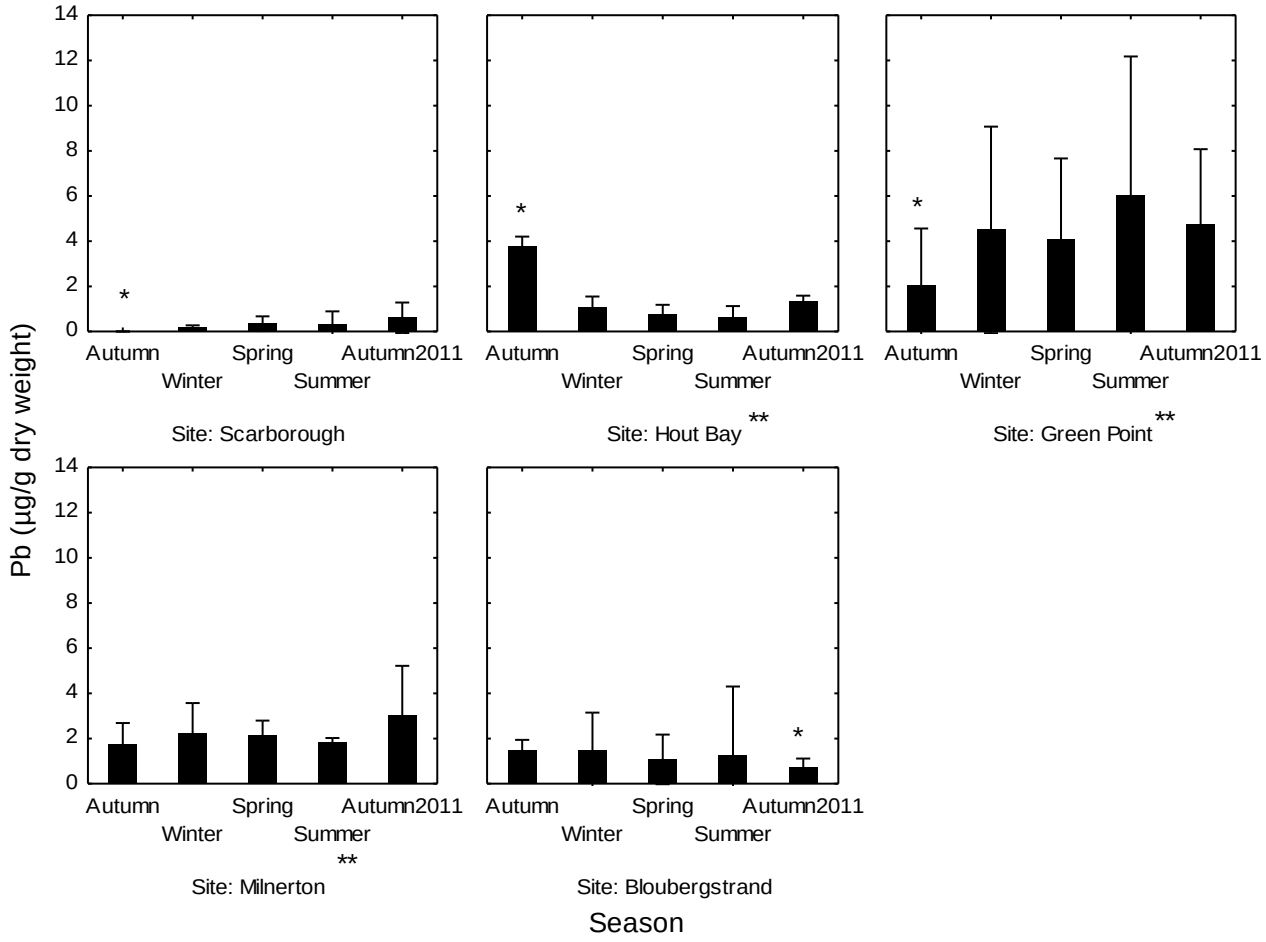

Figure 3-15. Mean Pb concentrations (µg/g dry weight) (±SE) (n=8) measured in water grouped per season and categorized per site from autumn 2010 to autumn 2011. * indicates significant differences between seasons at that site using the Tukey Honest Significant Difference post-hoc comparison test ($p<0.05$). ** indicates significant differences from Scarborough using the Dunnett's post-hoc test ($p<0.05$).

Chapter 4

THE BIOACCUMULATION OF METALS IN *MYTILUS GALLOPROVINCIALIS* OFF THE WEST COAST OF THE CAPE PENINSULA, SOUTH AFRICA

4.1. Introduction

There is a growing concern about the accumulation of detrimental contaminants in the coastal environment (water and sediments) due to their ability to bioaccumulate in organisms and subsequent adverse effects on the biota (Morse *et al.*, 1993; Besada *et al.*, 2011). These contaminants include metals that are natural components of the marine environment (Ansari *et al.*, 2004; Besada *et al.*, 2011). The sources of the metals are varied and include freshwater (Forget, 2003) and atmospheric sources (Williams *et al.*, 1998) as well as anthropogenic discharges (Goldberg & Bertine, 2000). The continual release of metals has resulted in concern about the integrity of coastal environments, in particular those with high tourism revenues (Goldberg & Bertine, 2000; Beaumont *et al.*, 2007; Besada *et al.*, 2011), as polluted environments could result in decreased revenue (Beaumont *et al.*, 2007).

The effects of metals in the environment are often not evident until large scale effects such as changes in ecosystem structure and function can be detected (Cevika *et al.*, 2008). By then the metals could have accumulated in organisms (as well as the food chain) (Besada *et al.*, 2011) and this results in people consuming seafood that has been exposed to metals, with potential dire consequences to their health (Cevika *et al.*, 2008; Joksimovic *et al.*, 2011). Knowledge about the levels of metals within systems are therefore important as these could provide information that could prevent people suffering from exposure to detrimental levels of metals and provide information about the toxicity of organisms prevalent in the ecosystem. Determination of metal concentrations in organisms should therefore be part of any assessment and monitoring programme of coastal systems (Shulkin *et al.*, 2003).

Marine bivalves are known to accumulate metals to relatively high concentrations in their tissue and shells without apparent negative physiological effects (Regoli *et al.*, 1991). This therefore makes them ideal organisms to monitor the levels of metals in the marine environment (Goldberg, 1975; Goldberg, 1986; Shulkin *et al.*, 2003) as they have been recorded to accumulate metals by factors of 10^2 to 10^5 (Besada *et al.*, 2011). Mussels are cosmopolitan and easily collectable in coastal areas without significantly affecting their populations (Goldberg & Bertine, 2000). To promote a uniform and general approach to marine pollution monitoring, Goldberg (1975) proposed a global monitoring programme using mussels as indicator organisms. This monitoring programme, called the Mussel Watch Programme (MWP) has been functional internationally since 1976 (Goldberg *et al.*, 1983) and in South Africa since 1985 (Kiviets, 2010). No publications about the levels of metals in the mussel *M. galloprovincialis* have been published in the last 20 years. Given that Cape Town is a major tourist destination (Anon, 2008) and has one of the busiest ports in the country, it is important to know whether metals in the environment are bioavailable as well as what the levels of metals in mussels are. This in turn will determine whether metals in *M. galloprovincialis* are toxic for human consumption (pose health risks) and if they could be accumulating metals that in turn could affect the health of the coastal ecosystem.

The objective of this study was to determine the bioaccumulation of iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in *Mytilus galloprovincialis* at five sites along the west coast of the Cape Peninsula, Cape Town.

4.2. Materials and Methods

4.2.1. Mussel sampling

Specimens of *Mytilus galloprovincialis* were collected seasonally between March 2010 and March 2011, during the months of March 2010 (autumn 2010), July (winter 2010), September (spring 2010), January (summer 2010), and March 2011 (autumn 2011). Samples were collected at spring 2010 low tide, with sampling always commencing at Scarborough, the southern-most site. Refer to Chapter 3 for information about the five sampling sites (Table 3-1; Figure 3-1). Most sampling

commenced at approximately 09h00. Samples were collected randomly at the five sites. Approximately 15 mussels of the same size range (40 - 70 mm) were collected randomly at each site and placed in plastic bags. Since body weight may influence variability of metal concentrations (Bourgoin, 1990), mussels of similar length were chosen as these were of the same age class. The mussels were then placed in cooler boxes whilst being transported to the laboratory. Once in the laboratory, the specimens were frozen at -18ºC until the analyses were done.

4.2.2. Metal analysis

Metal analysis was done according to the method of Odendaal and Reinecke (1999). Frozen mussel samples (n=8) were defrosted and oven dried for 48 hrs at 60° C in a Memmert drying oven. The soft tissue and shells were weighed and separated before oven drying. The dried tissue samples were weighed and homogenized per individual mussel with a mortar and pestle. Sub-samples of individual mussels (± 0.2 g) were digested using 10 mL of nitric acid (HNO_3). Samples were then heated to 40 °C in a Grant UBD heating block for one hour, thereafter to 120 °C for 3 hours. The digestates were allowed to cool and then filtered through a Whatman No. 6 filter paper and then through a 0.45 µm membrane micro-filter (Millipore) paper using a needle and syringe. Samples were then placed in plastic centrifuge tubes containing 5 mL digestate and 10 mL distilled water and stored in a refrigerator until further analysis was done. A blank accompanied all samples when analyses of samples were done. The concentrations of manganese (Mn), Iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) was analysed, with 7-8 replicates being done for each metal using the Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS). Concentrations of metals are presented as µg/g dry weight.

The ICP results were calculated as follows:

$$\text{metal} = \left[\frac{\text{ICP reading} - \text{blank}}{\text{mass (g)}}\right] \times \text{dilution factor (20)}$$

All metal concentrations in *M. galloprovincialis* were expressed as µg/g dry weight.

4.2.3. Statistical analysis

All calculations and data analysis were done using Statistica v10 (Statsoft). The one way ANOVA was used to determine whether there were seasonal and spatial differences in mean metal concentrations in *M. galloprovincialis*. The data were tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests respectively, prior to post hoc comparisons. Data that did not meet the requirements for parametric tests were log transformed. Tissue metal concentrations were analysed using a 2-way ANOVA with season and site as independent factors. Post-hoc comparisons were made using Bonferroni's test to determine if there were significant differences between sites and seasons ($p<0.05$). The Dunnet post hoc test was used to determine significant differences between Hout Bay, Green Point, Milnerton, Bloubergstrand and Scarborough (control site). Non-parametric Spearman rank correlations of metals in *M. galloprovincialis* were determined.

To investigate the similarity in metals at different sites and seasonally, dendrograms representing cluster analysis of the six metals were produced using PRIMER (Plymouth Routines in Multivariate Ecological Research) V6 was used. PRIMER is a software package that consists of a wide range of univariate, graphical and multivariate routines for analysing arrays of data sourced from community ecology (Clarke & Gorley, 2006). The software has a range of applications including analysis of biological assemblages, but more specifically for this study, application to ecotoxicology by considering concentrations of metals in mussels. The principle application of the software is that data sets are reduced to a triangular matrix representing the (dis) similarity of every pair of sample in terms of their assemblages. Clustering and ordinations techniques can then be applied to display the relationship between samples (Clarke & Gorley, 2006).

A multi-dimensional scaling (MDS) ordination of the concentrations of the metals measured in *M. galloprovincialis* from all the sites for the entire study (March 2010 to March 2011) was produced. Data were fourth root transformed and Euclidean distance used to produce a resemblance matrix.

Dendrograms representing the cluster analysis of samples were produced to illustrate groups of samples that were similar to each other. According to Clarke & Gorley

(2006), samples within a group are considered more similar than those from other groups. Groupings were arbitrary as distinct similarities were difficult to ascertain as no “good” similarity or distance is provided. All data were log x + 1 transformed, normalised and Euclidean distance used to produce a similarity matrix. Transformation of the environmental data was done to justify the Euclidean distance as a dissimilarity measure on normalised data (Clarke & Gorley, 2006). According to these authors, Euclidean distance is considered an appropriate measure of environmental data.

The efficiency of metal accumulation in *M. galloprovincialis* was evaluated by calculating the biosediment accumulation factor (BSAF). The BSAF is defined as the ratio between the metal concentrations in the organism to that in the sediment (Lau *et al.*, 1998).

4.3. Results

4.3.1. Metal concentrations in *Mytilus galloprovincialis*

4.3.1.1. Iron

The mean concentrations (±SE) of Fe measured in the soft tissue of *Mytilus galloprovincialis* are illustrated in Figure 4-1. The mean Fe concentration recorded for the study period was 116.93 ± 212.66 µg/g dry weight with the highest recorded concentrations being 2075.18 µg/g at Green Point during spring 2010. Generally, the Fe concentration values ranged between 0.01 ± 0.003 µg/g at Scarborough during autumn 2010 and 627 ± 162 µg/g dry weight at Hout Bay during autumn 2010. At Scarborough and Green Point, the Fe concentrations were significantly lower in autumn 2010 than the other seasons and from winter 2010 to autumn 2011, the Fe concentrations did not differ significantly at these two sites ($p<0.05$). At Hout Bay the Fe concentrations decreased significantly from autumn 2010 to winter 2010 as well as from winter 2010 to spring 2010 ($p<0.05$). There were no significant differences in Fe concentrations from spring 2010 to autumn 2011 at Hout Bay. At Milnerton and Bloubergstrand, Fe concentrations decreased significantly from autumn 2010 to winter 2010 and then increased significantly during spring 2010 ($p<0.05$). From

spring 2010 onwards, the Fe concentrations decreased at both Milnerton and Bloubergstrand, but only at Bloubergstrand was the decrease significant ($p<0.05$). The Fe concentrations from summer 2010 to autumn 2011 did not differ significantly for Milnerton and Bloubergstrand ($p>0.05$). The Dunnet's post-hoc ANOVA test indicated that Fe concentrations at Hout Bay, Green Point, Milnerton and Bloubergstrand were significantly different from Scarborough ($p<0.05$).

4.3.1.2. Manganese

The mean Mn concentration in the soft tissue of *M. galloprovincialis* for the study period was 2.30 ± 3.30 µg/g dry weight, with the highest value recorded in spring 2010 at Green Point 16.05 µg/g (Figure 4-2). Mean manganese values ranged between 0.0003 ± 0.00009 µg/g in autumn 2010 at Scarborough and 9.38 ± 2.94 µg/g dry weight in Hout Bay during autumn 2010. At Scarborough the Mn concentrations were significantly lowest in autumn. From winter 2010 to autumn 2011, there were no significant differences in Mn concentrations at Scarborough ($p>0.05$). At Hout Bay, Mn concentrations decreased from autumn 2010 to spring 2010, but only the decrease from winter 2010 to summer 2010 was significant ($p<0.05$).

Although Mn concentrations increased in autumn 2011, the increase was not significant ($p>0.05$). At Green Point the Mn concentrations increased significantly from autumn 2010 to winter 2010. From spring 2010 onwards, the Mn concentrations deceased, although the decrease was not significant from one season to the next. However, the decrease in Mn from winter 2010 to summer 2010 was significant at Green Point ($p<0.05$). At Milnerton, the Mn concentrations were significantly higher in autumn 2010, spring 2010 and autumn 2011 ($p<0.05$). At Bloubergstrand, Mn concentrations decreased significantly from autumn 2010 to winter 2010. Although the Mn concentrations increased from spring 2010 onwards, the increases were not significant ($p>0.05$). The Dunnet's post-hoc ANOVA test indicated that Hout Bay, Green Point, Milnerton and Bloubergstrand Mn concentrations were significantly higher than at Scarborough ($p<0.05$).

4.3.1.3. Copper

Mean Cu concentrations for the study period autumn 2010 to autumn 2011 was 4.95 ± 8.61 µg/g dry weight (Figure 4-3). The highest Cu concentration was recorded at Green Point during spring 2010 (55.48 µg/g). The mean range of Cu concentrations was 0.003 ± 0.00008 in Scarborough during autumn 2010 and 22.04 ± 6.98 µg/g in Milnerton during winter 2010. At Scarborough, mean Cu concentrations were significantly lower in August 2010 than the other seasons. From winter 2010 to autumn 2011, Cu concentrations did not differ significantly at Scarborough ($p>0.05$). At Hout Bay, the Cu concentrations did not differ significantly for the entire study period. At Green Point, the Cu concentrations increased significantly from autumn 2010 to winter 2010. From winter 2010 onwards, the Cu concentrations remained constant, with no significant differences recorded from one season to the next. However, the autumn 2011 Cu concentrations differed from autumn 2010 ($p<0.05$). At Milnerton and Bloubergstrand, Cu concentrations were the lowest in autumn 2010, increased significantly in winter 2010 and then decreased toward spring 2010. Only the decrease at Bloubergstrand was significant ($p<0.05$). For both Milnerton and Bloubergstrand, the Cu concentrations from spring 2010 to autumn 2011 did not differ significantly ($p>0.05$). The Dunnet's post-hoc ANOVA test indicated that Hout Bay, Green Point, Milnerton and Bloubergstrand Cu concentrations were significantly different from that of Scarborough ($p<0.05$).

4.3.1.4. Zinc

Figure 4-4 illustrates the mean Zn concentrations recorded in *M. galloprovincialis* for the study period autumn 2010 to autumn 2011 where the mean Zn concentration recorded was 70.15 ± 139.26 µg/g dry weight. The highest Zn concentration in the mussel *M. galloprovincialis* was recorded in spring 2010 at Bloubergstrand (1150.35 µg/g). The mean spatial records of Zn indicated that the lowest mussel Zn concentrations were found at Scarborough (0.007 µg/g, ± 0.002) and the highest mean Zn concentrations in the mussel was at Bloubergstrand in spring 2010 (416.19 ± 363.01 µg/g). At Scarborough, Zn concentrations increased significantly from autumn 2010 to spring 2010. Although Zn decreased from spring 2010 to summer

2010 and then increased in autumn 2011, the changes were not significant ($p>0.05$). At Milnerton Zn concentrations decreased from autumn 2010 to spring 2010 and then increased again towards autumn 2011. Although the changes in Zn concentrations were not significant form one season to the next, there was as a significant decrease from autumn 2010 to spring 2010. At Green Point the Zn concentrations were significantly lowest in autumn 2010. The Zn concentrations did not differ significantly for the remainder of the study period. At Milnerton Zn concentrations decreased significantly from autumn 2010 to winter 2010 ($p<0.05$). The Zn concentrations then increased towards autumn 2011, although the changes form one season to the next was not significant. At Bloubergstrand the Zn concentrations were significantly higher in autumn 2010 and spring 2010. The Zn concentrations in summer 2010 and autumn 2011 were significantly higher than winter 2010 and were significantly lower than autumn 2010 and spring 2010 ($p<0.05$). The Dunnet's post-hoc ANOVA test indicated that Hout Bay, Green Point, Milnerton and Bloubergstrand Zn concentrations were significantly different from that of Scarborough ($p<0.05$).

4.3.1.5. Cadmium

Cadmium concentrations were relatively high at most sites during the study period as depicted in Figure 4-5. The mean Cd concentrations for the study period were 5.06 (± 22.18) µg/g dry weight. The lowest mean Cd concentration was recorded at Scarborough during autumn 2010 (0.0004 ± 0.00008 µg/g) and the highest at Green Point during autumn 2010 (112.75 ± 64.19 µg/g). Cadmium concentrations in mussels at Scarborough increased significantly from autumn 2010 to spring 2010, decreased significantly again in summer 2010 and then increased significantly in autumn 2011 ($p<0.05$). At Hout Bay, the Cd concentrations were significantly higher in autumn 2010 and autumn 2011. At Green Point, the Cd concentrations decreased significantly from autumn 2010 to winter 2010, remained constant from winter 2010 to summer 2010, before increasing (not significant, $p>0.05$) in autumn 2011. At Milnerton, the Cd concentrations decreased significantly from autumn 2010 to winter 2010 ($p<0.05$). Thereafter, the Cd concentrations did not differ significantly for the remainder of the period of the study. At Bloubergstrand, winter 2010 Cd concentrations were significantly lower than the other sites. Spring 2010 Cd

concentrations were significantly higher than autumn 2010, summer 2010 and autumn 2011 ($p<0.05$). The Dunnet's post-hoc ANOVA test indicated that Hout Bay Cd concentrations were significantly different from that of Scarborough ($p<0.05$).

4.3.1.6. Lead

Lead concentration in *M. galloprovincialis* was relatively low throughout the study period (Figure 4-6). The mean Pb concentration in *M. galloprovincialis* sampled in the present study was 5.77 ± 55.49 µg/g dry weight. The high variation recorded was due to a very high concentration (758 µg/g) being reported in autumn 2010. The lowest mean Pb concentrations were recorded at Scarborough during autumn 2010 (0.0001 ± 0.00002 µg/g) and the highest at Green Point during autumn 2010 (143.23 ± 301.72 µg/g). Pb concentrations per site were relatively low and no clear patterns of seasonal changes evident. At Hout Bay and Green Point, Pb concentrations decreased from autumn 2010 through to spring 2010, with the changes being significant at Green Point ($p<0.05$). At Milnerton and Bloubergstrand, Pb concentrations decreased form autumn 2010 to winter 2010 and then increased again towards spring 2010. The Dunnet's post-hoc ANOVA test indicated that Hout Bay, Green Point, Milnerton and Bloubergstrand Pb concentrations were significantly different than Scarborough ($p<0.05$).

4.3.2. Summary of environmental variables

Metal concentrations were correlated using non-parametric Spearman Rank Order correlations and the results revealed that all metals had significant positive relationships (Table 4-1). Metals were significantly negatively correlated with season suggesting that metal concentrations decreased from autumn 2010 to autumn 2011. There were no significant relationships between metals and site.

An MDS (multi-dimensional scaling) of seasonal data for all the sites and metals combined (stress = 0.11) showed that there were overlaps between the seasonal samples. However, autumn 2010 and winter 2010 samples showed two groups whereas spring 2010 data were scattered (Figure 4-7). Both summer 2010 and

autumn 2010 data were closely grouped. The MDS of spatial data (per site) (stress = 0.11) indicated that Hout Bay and Green Point were the most scattered (Figure 4-8). Milnerton and Bloubergstrand indicated that two groups (with some overlap) were present at these sites. Scarborough data were closely grouped (Figure 4-8). Generally there was an overlap of data at all sites, suggesting that the data were closely associated, albeit that the clusters were not sharply defined. An Analysis of Similarity (ANOSIM) between the seasons showed that there was no significant difference between seasonal metal concentrations (Global R = 0.24, $p > 0.05$). An ANOSIM between the five sampling sites also indicated no significant difference between sites and metal concentrations (Global R = 0.156, $p > 0.05$).

The results of the seasonal similarity percentages (SIMPER) analysis (Table 4-2) showed that during autumn 2010 and winter 2010, most metals (except Cu during autumn 2010) were responsible for the similarity at all sites. During spring 2010, all metals, except Cd and Pb, were responsible for the similarities within the mussel metals concentrations. The results of the spatial SIMPER analyses (Table 4-3) indicated that all metals, except Pb were responsible for the similarity at Scarborough and Milnerton. At Hout Bay, Fe, Zn and Pb were responsible for similarities. At Green Point, Pb and Cd was the largest contributor to similarities. At Bloubergstrand, Fe, Cu, Zn and Cd were the main contributors to similarity.

Although the MDS showed some separation between seasonal and spatial metal data, these results were not conclusive (Fig 4-7 and 4-8). The dendrogram representing the cluster analysis of metals between the different sites (Fig 4-9) suggested that there was some segregation of the metal data. Samples from Scarborough were more distinct than the rest of the sites. The dendrogram for seasonal cluster analyses revealed poor grouping of data (Figure 4-10) and high variability, suggesting that metal contamination was not influenced by seasonal variations. Table 4-1, however, indicated that there was a significant negative correlation between season and metals.

4.4. Discussion

Mussels are considered suitable for biomonitoring studies due to the biological responses these organisms have to contaminant exposure and as a consequence

are being used more often in environmental quality evaluations and risk assessments (Giarratano *et al.*, 2010). The reason for their suitability is that they can accumulate various elements (Regoli, 1998). Other reasons for mussels being ideal biomonitors are that they are considered to have suitable dimensions, are abundant in an ecosystem and accumulate elements to a degree of suitable measure (Goldberg *et al.*, 1983).

The Mediterranean mussel, *Mytilus galloprovincialis,* is a predominantly sublittoral mussel prevalent along the coasts of southern Africa. These mussels can densely cover rocky shores up to nearly 1 m above the low water spring tide (Du Plessis, 1977; Griffiths, 1981). These mussels form extensive beds in the lower intertidal and subtidal zones, in particular along the west and southwest coast of South Africa (Griffiths, 1981). The distribution of *M. galloprovincialis* is from Walvis Bay in Namibia to the north, to Port Alfred along the south coast of South Africa (Branch *et al.*, 2010). Over the past 30 years, *M. galloprovincialis* has become increasingly dominant as an alien mussel, often displacing the local species, *C. meridionalis,* due to its ability to tolerate a wider range of environmental conditions (Griffiths, 1981). This dominance could also be because *M. galloprovincialis* are superior bioaccumulators of many chemical substances and they are therefore able to tolerate exposure to pollutants such as metals (Cevik *et al*., 2008). Although extensive studies have been done on metal accumulation in mussels in other parts of the world, in particular the northern hemisphere (Sarki *et al.*, 1995; Cajaraville *et al.*, 2000; Cevik *et al.*, 2008; Ünlü *et al.*, 2008; Besada *et al.*, 2011), such investigations are sparse in the southern hemisphere and can be considered to be non-existent in South Africa since the 1980's (O' Donoghue & Marshall, 2003).

The present study has shown, compared to the MWP data, that all the metals investigated, except Zn, have higher mean concentrations than MWP data from 1985 to 2008 for the same area (Chapter 2). As such, the MWP data are considered to be background levels for this study, albeit that the concentrations for the present study were not indicative of pollution levels (Table 4-4).

The order of mean concentrations (µg/g dry weight) of metals (for all sites combined and for the entire sampling period) in *M. galloprovincialis* was Fe (116.93 µg/g) > Zn (70.14 µg/g) > Pb (5.77 µg/g) > Cd (5.06 µg/g) > Cu (4.95 µg/g) > Mn (2.30 µg/g).

The spatial and temporal variations of metal concentrations of this study were difficult to interpret due to high variability, but generally, metal concentrations in the tissue of *M. galloprovincialis* were lowest at Scarborough, with the lowest significant ($p<0.05$) concentrations recorded in winter 2010. Given that the study was conducted in a winter rainfall area, the low concentrations recorded is indicative of either flushing of the system or that the area is not influenced by anthropogenic sources of Pb. Metals such as Fe, Cu, Zn and Mn are considered essential elements since they play a role in the biological functioning of organisms (Cevik *et al*., 2008). In order to minimise the effects of metals, various authorities have set metal contamination guidelines/standards for mussels to indicate whether they are contaminated (Table 4-5). Consumption of essential elements such as Fe and Cu is permissible, but only to certain concentrations. However, metals such as Pb and Cd are non-essential, as they are toxic even at trace concentrations (Matta *et al.*, 1999; Cevik *et al.*, 2008).

Cantillo (1998) provided a dataset of all MWP's from across the world. There were some locations where no data was available which resulted in the author not being able to report any worldwide temporal trends. Interestingly, despite having data from across the world, the author was not able to provide an independent basis for declaring any specific metal concentrations as a natural acceptable level, but suggested the lowest 85th percentiles from National Status and Trends (NST) data as well as French MWP data as indicative of contamination (*i.e.*, elevated by human activity). The results are presented in Table 4-5.

Over the past 15 years, investigations of marine pollution by analysing metal concentrations in mussels and sediments have intensified, particularly in Europe (Joksimovic *et al*., 2011). Sediments are considered to be contaminant sinks as pollutants and can remain in areas for lengthy periods (Schiff, 2000). As such, metals that accumulate in sediments, can over time, be transferred to and potentially accumulate in marine organisms. According to Rainbow & Phillips (1993), marine organisms have the ability to accumulate high levels of metals from their environment, in particular from sediment. Although most sediment-adsorbed contaminants are not readily available to aquatic organisms, variability within the water may result in the release of metals back into the water, making sediments an important source of pollution in certain environmental conditions (Rainbow & Phillips,

1993; Burger & Gochfeld, 2006). Metal concentrations in mussels are known to correlate with metal levels in proximate sediments (Puente *et al*., 1996). Hence data concerning metal concentrations in sediment is essential to assess their potential to accumulate in marine organisms (Joksimovic *et al*., 2011).

Data from sediment samples (Chapter 3) were correlated with metal concentrations in mussels sampled from the same site (Table 4-6). Spearman rank correlations indicated significant positive correlations between Fe, Mn and Pb concentrations in sediments and mussels sampled. There was a significant negative correlation between sediment and mussel Cu concentration. There were no significant correlations between Zn and Cd sediment and mussel correlations. These results suggest that there is no uniform accumulation of metals in mussels from the surrounding medium and levels of metals within mussels are subject to the bioavailability of metals and these in turn are influenced by the mussel's physiology to take up the metals (Eisler, 1981; Bayne, 1986; Almeida *et al*., 2007).

Metal bioavailability has traditionally been defined to include the availability of metals to organisms as well as the availability of metals to tissues within organisms once inside the organism (Peakall & Burger, 2003). According to Mountouris *et al.,* (2002) metal bioavailability results in high concentrations of the corresponding metal in biota through a bioconcentration process. Bioconcentration is the process by which a chemical species is accumulated into organisms from its surrounding medium (Mountouris *et al.,* 2002) and is calculated using the BSAF (also termed the bioconcentration factor). Bioavailability is affected by sediment characteristics (Mountouris *et al.,* 2002) such as: the concentration of iron, manganese and aluminium oxides (Bendell-Young & Harvey, 1991, Shea, 1988), levels of organic carbon (Mahoney *et al*., 1996) and acid volatile sulphide (Chapman *et al.,* 1998). Bioavailability is also dependant on several environmental factors: physical (grain size of the sediment and suspended particulate materials), chemical (solubility, reactivity of compounds, complexing agents), and biological (benthic or pelagic organisms, modes of exposure) (Geffard *et al*., 2003). The uptake of metals is also highly dependent on geochemical factors such as temperature, pH and dissolved oxygen (Lu *et al.*, 2005).

The efficiency of metal accumulation in *M. galloprovincialis,* as evaluated by calculating the biosediment accumulation factor (BSAF), is indicated in Table 4-7 and can be indicative of the bioavailability of the metals sampled. The orders of BSAF were: Cd > Zn > Pb > Cu > Mn > Fe. The results indicated that Cd, Zn, Pb and Cu had the highest mean BSAF, as the metal concentrations were higher in the mussels than the sediment, suggesting that these metals were bioavailable for uptake. The lowest BSAF were calculated for Fe and Mn. The low BSAF values suggest that both Fe and Mn were either not bioavailable, the mussels were able to regulate the metals effectively or that physiological processes prevented the metals from being accumulated. These results are higher than the BSAF reported by Abdallah & Abdallah (2008), who recorded BSAF values in bivalves for Cd (1.26), Zn (1.43), Cu (0.32), Mn (0.24) and Fe (1.33). The BSAF values in the present study are considerably higher than that reported by Abdallah & Abdallah (2008) (except for Mn and Fe): Cd (17.36), Zn (6.32), Cu (3.59), Mn (0.49), and Fe (0.32). The higher BSAF values reported in the present study are hence indicative of the high bioavailability of metals in the region. An understanding of the bioavailability of metals is thus important to consider when investigating whether *M. galloprovincialis* in the Western Cape are ideal biomonitoring tools of metal pollution. Viarengo & Canesi (1991) found that *M. galloprovincialis* was able to rapidly eliminate Cu from its system, but was less able to expel Cd. These results could therefore explain the higher mean Cd BSAF values recorded in the mussels as they are not able to eliminate Cd effectively from their tissue.

The results of the present study, together with the results of Chapter 3 have indicated that metal concentrations in the sediment were bioavailable to be accumulated in *M. galloprovincialis*. However, there are various factors pertaining to *M. galloprovincialis* that affect the bioavailability of metals they are exposed to. Such factors can be defined by attributes of the individual mussel, groups of mussels or the species as a whole that can influence the amount and degree of metal exposure, uptake, absorption, biokinetics, susceptibility and toxicity (Burger *et al.,* 2003). According to these authors, such factors include age, gender, size and weight, nutritional status, genetics and behaviour which influence exposure.

Iron had the highest metal concentrations in mussels sampled. The Fe concentrations from this study were higher than *C. meridionalis* sampled in Saldanha Bay in the 1970's (Watling & Watling, 1976). These authors recorded Mn values of 60 µg/g dry weight in *C. meridionalis* in Saldanha Bay. The mean Mn concentration of this study was 116.93 µg/g dry weight. Orren *et al.,* (1980) reported low Fe concentrations in Bloubergstrand from samples collected in 1980, 17.4 µg/g dry weight in winter and 59.3 µg/g dry weight in late spring. One contributing factor to the higher sources of Fe was increased anthropogenic sources of runoff from urban and industrial sources. However, the low BSAF value for Fe (0.14) suggests that *M. galloprovincialis* is either able to control the levels of Fe in its tissue or the metal is not bioavailable. Iron is an essential trace metal that is required for development and its uptake is known to be highly specialised due to complex chemical speciation linked to intracellular homeostasis (Worms *et al*., 2006). According to these authors, most bioavailable iron in the natural environment is strongly associated to poorly characterised organic ligands. Furthermore, studies have indicated that iron complexes and colloidal iron are accessible to organisms and that bioavailability is enhanced by both chemical and biological mechanisms (Allison *et al.,* 1998; Peakall & Burger, 2003; Worms *et al*., 2006).

The Mn concentrations from this study were lower than historical data of *C. meridionalis* sampled in the same region (Watling & Watling, 1976). These authors recorded Mn values of 9 µg/g dry weight in *C. meridionalis* in Saldanha Bay. The mean Mn concentration of this study was 2.3 µg/g dry weight. The reason for the lower Mn concentrations than that of Watling & Watling (1976) is uncertain and requires further investigation. The Mn concentrations recorded in South Africa (historical and from this study) were lower than those reported in the Mediterranean (22.67 µg/g dry weight) (Abdallah & Abdallah, 2008). According to Abdallah & Abdallah (2008), variations in Mn concentrations, are dependent on the feeding habit, age, size and length of organisms and their habitats. Alison *et al*. (1998) fed *M. edulis* various ranges of permanently suspended particulate matter (PSPM) (particles ranged from <3 to 40 µg) and found that Mn in the pseudofaeces were often significantly higher than that in the PSPM , indicating that the mussels had rejected the particles enriched with this metal. The exact mechanism is uncertain but the

concentration of organic materials excreted in the pseudofaeces suggested that the organic materials were ingested preferentially (Alison *et al*., 1998).

Copper is an important trace element. It is a component of hormones, vitamins, enzymes as well as nucleoprotein complexes. On the other hand, it is also a biocide, and because of this element of toxicity, it is regulated by living organisms (Phillips, 1977). The mean Cu concentration in *M. galloprovincialis* for the study period (4.95 µg/g) was below the recommended limit for Cu (10 µg/g) as suggested by Cantillo (1998). However, Hout Bay (winter 2010), Milnerton (winter 2010 and spring 2010) and Bloubergstrand (winter 2010) Cu levels in mussels were above the recommended limit (Table 4-4 and 4-5). This suggests the sources of Cu in the study area were from runoff, given that the region has its highest rainfall during winter, which in turn could have made Cu bioavailable. The legal limit of Cu allowed in shellfish in South Africa is 50 µg/g (South Africa, 1994). According the results of this study, none of the sites reported Cu concentrations above the legal limit.

The Cu concentrations in the present study were lower than that reported by Watling & Watling (1976), who recorded Cu concentrations ranging between 7 and 14 µg/g dry weight in *C. meridionalis*. Copper is a naturally occurring element and essential for cellular metabolism. The uptake of Cu is hence variable, dependant on the kinetics of uptake and excretion (Philips, 1976). The low Cu concentrations in *M. galloprovincialis* recorded in the present study could be indicative of *M. galloprovincialis* not accumulating Cu in its soft tissue because Cu was either not bioavailable or that the mussel was able to regulate Cu. The BSAF (1.81) suggests that the mussel was able to regulate Cu and that Cu was bioavailable. Allison *et al*. (1998) reported that Cu concentrations in the faeces of *M. edulis* was significantly lower than in the particulate matter it was fed, reflecting a net loss of Cu from particles in the mussel gut. It was suggested that changes to the digestive physiology of mussels (increase in the proportion of food sent to the digestive gland and increase in gut passage time) compensated for lower digestion rates. These alterations increased the net release of particulate Cu and hence the bioavailability of Cu to the mussel. According to Cevik *et al.* (2008) Cu is a critical element to measure when analysing pollution of an area as it is both an important trace element for physiology but can also affect the health of organisms if available to bioaccumulate.

The mean Zn concentrations (spatial and temporal) for the study period (70.15 µg/g) were below the recommended level (200 µg/g) suggested by Cantillo (1998). However, concentrations > 200 µg/g were recorded at Green Point as well as at Bloubergstrand (autumn 2010 and spring 2010) (Figure 4.4). The legal limit of Zn allowed in shellfish is 300 µg/g (South Africa, 1994). According to the results of the present study, Zn concentrations in the soft tissue of mussels from Bloubergstrand were above the permissible concentrations during spring 2010. According to Cevik *et al.* (2008), Zn is a critical element to measure when analysing pollution of an area. Watling & Watling (1976) recorded Zn mussel concentrations in Saldanha Bay ranging from 73 to113 µg/g dry weight (mean = 95.7), higher than that recorded during this investigation but this could have been due to higher environmental concentrations and/or increased Zn bioavailability.

The mean Cd concentrations from the present study (5.06 µg/g) were above the recommended value (3.7 µg/g) proposed by Cantillo (1998). The high mean concentration was influenced by very high Cd concentrations recorded at Green Point during autumn 2010 (>100 µg/g). The permissible concentration of Cd in mussels is 3.0 µg/g (South Africa, 1994). The reasons for the high Cd concentrations are attributed to high ambient Cd concentrations in the sediment and water and the source of it was probable from the adjacent dense residential area and storm water pipe close to the sampling site. The results indicate the bioavailability of Cd for mussels, and could explain the high levels of Cd accumulated in the organism. Cadmium concentrations above 3.0 µg/g were recorded at Scarborough (winter 2010, spring 2010 and autumn 2011), Green Point (autumn 2010, spring 2010, summer 2010 and autumn 2011), Milnerton (spring 2010, summer 2010 and autumn 2011) and Bloubergstrand (autumn 2010 and spring 2010). A study by Topping (1973) reported higher concentrations of Cd in the hepatopancreas of arthropods than in mussels. The origins of the Cd were uncertain but it was proposed that the dominant pathway of Cd for the arthropods was via food rather than sea water. Since mussels are filter feeders and their food obtained from the sea water, mussels obtained their sources of Cd from both the sea water and prey within the sea water. A considerable amount of particulate cadmium comes from the burning of plastics products, pigments and rubber goods (Schroeder, 1974) but the high Cd levels recorded in the present study could not be indicative of burning of plastic and rubber

products at Green Point, Milnerton and Bloubergstrand as such activities do not occur in these area.

Irrespective of the source of Cd, it is its bioavailability and uptake that is important. Pan & Wang (2004) studied the influences of dissolved organic carbon (DOC) and colloidal organic carbon on the bioavailability of Cd in *Perna viridis*. These authors found that DOC from decomposed diatoms resulted in linearly increasing of Cd with increasing DOC content. The west coast of the Western Cape is situated in a major upwelling centre (Shannon, 1985). Upwelling intensifies in spring, with further upwelling events in autumn. The upwelling events during late summer and autumn results in algal blooms and in some cases the blooms result in red tides (harmful algal blooms) (Branch *et al*., 2010) which in turn may provide DOC from decomposing diatoms. The extraordinary high Cd concentrations in *M. galloprovincialis* at Green Point (112 µg/g dry weight) could have been from elevated diatoms present in the area. The mechanism underlying the increase of Cd uptake with increasing DOC is not exactly known, but the origins of DOC could have substantial influence on DOC-metal interaction in mussels (Pan & Wan, 2004).

The mean Pb concentrations in *M. galloprovincialis* for the entire study period (5.77 µg/g) as well as at Hout Bay during autumn 2010 and at Green Point during winter 2010 (Figure 4.6) were above the recommended contamination limit (3.2 µg/g) suggested by Cantillo (1998). A study by Watling & Watling (1976) reported Pb concentrations in *C. meridionalis* ranging from 2 to 5 µg/g (mean = 3.7 µg/g) in Saldanha Bay (approximately 150 km to the north of Cape Town), values that are lower than that reported in the present study. Higher concentrations than the permissible limits for human consumption of Pb (4.0 µg/g) (South Africa, 1994) were recorded at Hout Bay (autumn 2010) and Green Point (autumn 2010, winter 2010 and spring 2010). Giarratano *et al.* (2010) noted high Pb concentrations in mussels during their study and attributed it to Pb contamination from heavy shipping activity. This proposed reason could apply to Hout Bay, given that it has shipping traffic and shipyard activities. It is however unlikely to be the case that shipping activity be responsible for high Pb concentration in Green Point (under normal situations). The BSAF of 3.05 suggests that Pb was bioavailable in the area, most likely from the sediment. The winter 2010 sampling was undertaken during the 2010 soccer world

cup and two days before a match at the Cape Town Soccer stadium. Given the increase in shipping activity during the world cup, it could have been a contributing factor to increase Pb concentrations, given that the selected site was within a kilometre of the stadium and within 5 km of Table Bay harbour. The high Pb concentrations also correspond to high Pb concentrations in sediment at the same site (Figure 3.14, Chapter 3), further supporting the suggestion that the Pb was bioavailable. Another possible source of Pb is hence from the sediment in the area. Given that sediment are sinks for metals, it is possible that Pb from cars are prevalent in the sediment (Peakall & Burger, 2003). It is possible that upwelling could have provided a source of Pb from deeper lying areas (Preston, 2002). It is however more probable that stormwater runoff be the main contributor of Pb at Green Point. The source and metal composition (Pb speciation) from the stormwater requires further investigation. According to Goldberg *et al.* (1983), high concentrations of Pb in mussels that occur close to urban areas could be attributed to Pb alkyls as an anti-knock agent in fuels. This is unlikely to be the case here as Pb is no longer permissible in fuels since 2006 but still persistent though. It is therefore postulated that the Pb is from sewage, storm and river runoff (Goldberg *et al.*, 1983). Boyden (1977) reported that higher Pb was recorded in mussels from estuaries (hence areas with fresh water input). The results of the present study support this view as relatively low Pb concentrations were recorded for the duration of the study period.

There was a significant positive correlation between all metals measured in mussels (Table 4-1). This suggests that the correlated metals share a common accumulation process in tissue of mussels (Cevik, 2008). Results of the MDS and dendrograms suggest that the metal data are grouped (Figure 4.7 and Fig 4.8) albeit that the groups were not distinct (Figure 4.9 and Fig 4.10). The seasonal group that is discernable is winter 2010 (Figure 4.7 and Fig 4.10) and spatially, Scarborough (Fig 4.8 and Fig 4.9). Both the discernable groups could be attributed to anthropogenic sources of metals where, during winter 2010, there is a higher input of freshwater from rain (Shannon, 1985) and this could result in dilution effects of metals in the region but could also be a major contributor due to runoff of metals from the landward side of the coast. Wong *et al.* (2000) recorded significantly lower metal concentrations in mussels collected from Ma Liu Shui in Hong Kong. These authors attributed the lower concentrations to metal bioavailability, season and physiology.

The main contributing factor, however, was thought to be due to variation in water salinity and temperature, probably because it changes bioavailability. Higher temperature (due to less upwelling) and lower salinity (freshwater input) may have contributed to an increase in the filtration rate of mussels which could have resulted in more rapid accumulation of metals (Wong *et al.*, 2000). Scarborough is removed from anthropogenic sources of metals and hence has significantly lower metal concentrations in the environment and in the organisms than the rest of the study sites.

Despite variations between the study sites, the overall pattern at the respective sampling sites was similar for all sites combined. Hence, the order of magnitude of accumulation at all sites was Fe>Mn>Cu>Cd>Zn>Pb and reflects the trend at respective sampling sites. Both Fe and Mn are considered to be high probably due to natural sources and the high levels recorded are acceptable (Cevik *et al.*, 2008). However, the high Cu, Cd and Pb concentrations were at times higher than that permissible in South Africa with the mean concentration of Cd (8.71 µg/g) and Pb (9.93 µg/g) for all sites combined being higher than the permissible levels of 3 µg/g for Cd and 4 µg/g for Pb (South Africa, 1994).

The concept of bioavailability includes the accessibility of metals to tissues. According to Burger et al. (2003), by analogy, a chemical circulating in the blood may be more or less available to certain tissues, depending on binding to lipid solubility and transporters in particular cell types. This is a central issue, as toxicity in targeted cells is dependent on the presence of the metal. Assuming that the amount of metal which enters cells is equivalent to the effective dose or ambient concentrations, an indirect method which can be used in the study of metal accessibility to tissue is the assessment of antioxidant biomarkers. In the following chapter, antioxidant responses to various dosages of metals are investigated.

4.5. Conclusion

Biomonitoring can show temporal changes and the spatial distribution of local metal contamination. Mussels with higher concentrations of Cu and Zn in the soft tissue were found in areas with high metal loads in the sediment, especially at Milnerton

and Bloubergstrand. Metal concentrations in tissue of *M. galloprovincialis* did not follow similar patterns as sediment concentrations. Very high Cd concentrations in mussels off Green Point are indicative of Cd bioaccumulation and more investigations in bioavailability is needed. Further investigations that include changes in the distribution of metals between the dissolved and particulate phases are also needed. Crucial to understanding the importance of waterborne and dietary exposure routes of metals in their environment, is to be able to understand how metal accumulation takes place in these filter-feeding invertebrates (Giarratano et al., 2010).

Table 4-1: Results of the Non-parametric, Spearman Rank Order Correlations between all environmental variables measured showing r Values. * indicates significant r-values at $p < 0.05$.

	Site	Season	Fe	Mn	Cu	Zn	Cd	Pb
Site								
Season	-0.011							
Fe	0.081	-0.752*						
Mn	0.037	-0.786*	0.927*					
Cu	0.124	-0.763*	0.795*	0.847*				
Zn	0.056	-0.729*	0.901*	0.807*	0.724*			
Cd	0.039	-0.710*	0.846*	0.779*	0.652*	0.877*		
Pb	-0.133	-0.081*	0.929*	0.908*	0.789*	0.870*	0.874*	

Table 4-2. SIMPER (Similarity Percentage) of the seasons using all metal variables. A resemblance matrix using Euclidean distance was used in the analysis. The following table represents the average similarity between the seasonal groups. The values in bold represent the metals which contribute most to the similarity within each group.

Season	**Autum2010**	**Winter 2010**	**Spring 2010**	**Summer 2010**	**Autumn 2011**
Average squared distance	14.09	4.33	5.58	1.92	1.69
Environmental factor	**% contribution**	**% contribution**	**% contribution**	**% contribution**	**% contribution**
Fe	**25.02**	**9.38**	12.06	**23.71**	14.06
Mn	**14.38**	**22.79**	**23.39**	**18.79**	**24.68**
Cu	6.50	14.27	**29.96**	11.21	**19.90**
Zn	**13.04**	**17.82**	**31.42**	**24.47**	12.81
Cd	**20.57**	14.14	5.79	14.97	**28.55**
Pb	**20.49**	**20.60**	4.08	6.84	0

Table 4-3. SIMPER (Similarity Percentage) of the sites using all metal variables. A resemblance matrix using Euclidean distance was used in the analysis. The following table represents the average similarity between the five sampling sites. The values in bold represent the metals which contributed the most to the similarity within each group.

Season	**Scarborough**	**Hout Bay**	**Green Point**	**Milnerton**	**Bloubergstrand**
Average squared distance	6.54	2.57	8.10	2.88	3.47
Environmental factor	**% contribution**	**% contribution**	**% contribution**	**% contribution**	**% contribution**
Fe	**19.80**	**28.55**	**18.33**	**19.84**	**15.42**
Mn	**20.44**	9.61	5.66	**17.27**	7.59
Cu	**25.54**	7.32	7.83	**20.57**	**15.27**
Zn	**18.29**	**35.42**	**13.64**	**19.09**	**33.94**
Cd	**12.80**	4.12	**20.29**	**20.53**	**22.68**
Pb	3.13	**14.98**	**34.26**	2.69	5.10

Table 4-4. Mussel Watch Programme mean metal concentrations of Fe, Mn, Cu, Cd, Zn, and Pb (µg/g dry weight) in *M. galloprovincialis* along the west coast of the Cape Peninsula for the study period 1985 to 2008 for all sites combined.

	Mean	Maximum	Std. Dev.
Fe	40.3590	1309.000	109.1452
Mn	1.3343	64.700	3.9760
Cu	4.3826	101.000	4.9609
Cd	4.7209	39.100	5.1341
Zn	136.7960	1625.550	135.4597
Pb	3.4322	427.600	13.7640

Table 4-5. Concentrations of trace metals in mussels that is indicative of contamination (µg/g dry weight). Values for South Africa are permissible legal limits for shellfish.

Metal	Concentration		
	Global (FAO, 1983)	Black Sea (Cantillo, 1998)	South Africa (South Africa, 1994)
Fe	-	-	-
Mn	-	-	-
Cu	50-150	10	50
Cd	10	3.7	3
Zn	200-500	200	300
Pb	5-30	3.2	4

Table 4-6. Summary of Spearman rank correlations for combined metal concentrations in the sediment (S) (µg/g dry weight) and mussels (M) (µg/g dry weight) sampled at five sites along the west coast of the Cape Peninsula. Figures in bold are statistically significant at $p < 0.05$ level.

	M Fe	M Mn	M Cu	M Zn	M Cd	M Pb
S Fe	**0.348**					
S Mn		**0.314**				
S Cu			**-0.145**			
S Zn				-0.061		
S Cd					-0.115	
S Pb						**0.338**

Table 4-7. Mean concentration (µg/g dry weight) of metal in sediment and *M. galloprovincialis* as well as mean biosediment accumulation factor (BSAF).

	Mn	**Fe**	**Cu**	**Zn**	**Cd**	**Pb**
Sediment	4.66	835.69	2.73	14.26	0.29	1.89
M. galloprovincialis	2.3	116.93	4.95	70.15	5.06	5.77
BSAF	0.49	0.14	1.81	4.92	17.45	3.05

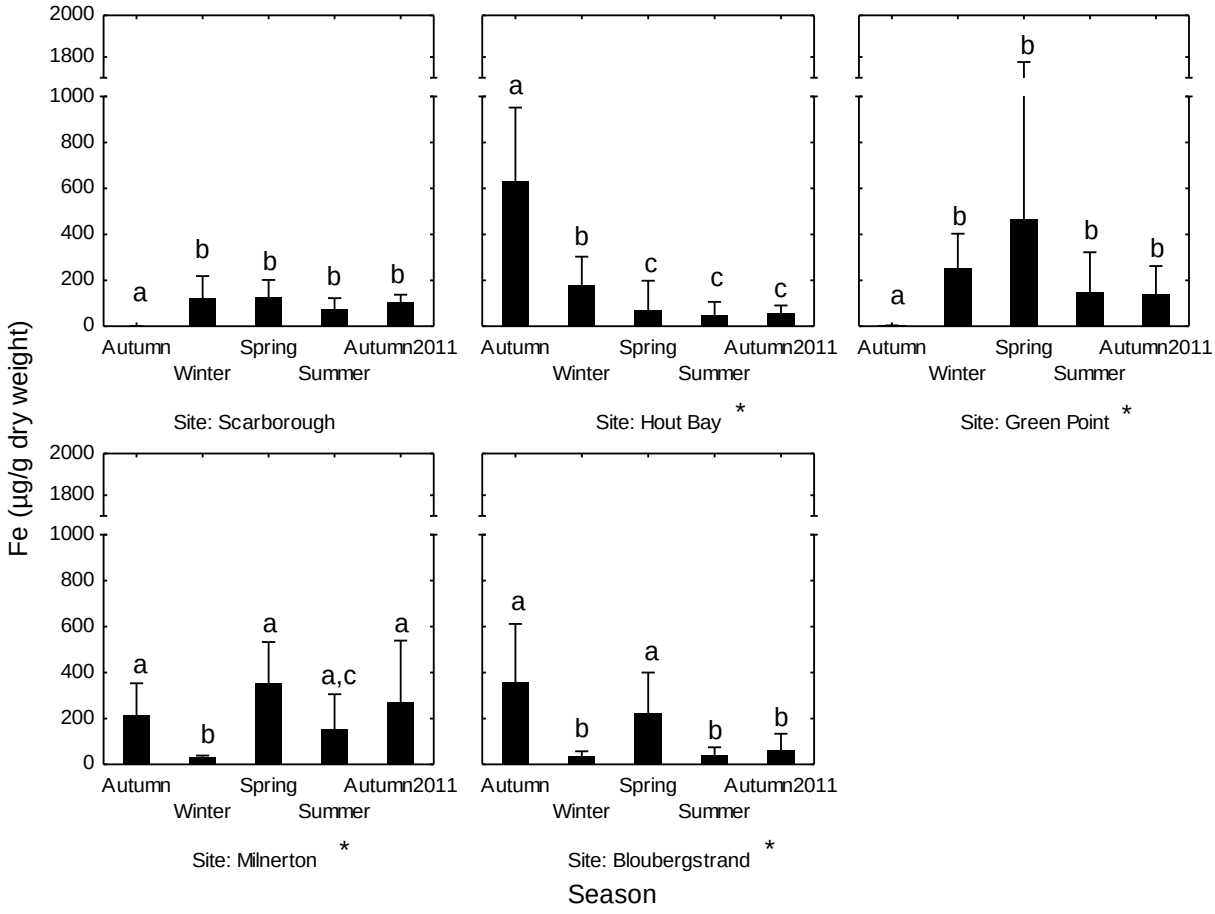

Figure 4-1. Mean Fe concentrations (µg/g dry weight) (± SE) (n=8) measured in *M. galloprovincialis* grouped per season and categorized per site from autumn 2010 to autumn 2011. Different letters indicates statistical differences between seasons using the Bonferroni post hoc test ($p<0.05$). * indicates significant differences from Scarborough (control site) using the Dunnet post hoc test ($P<0.05$).

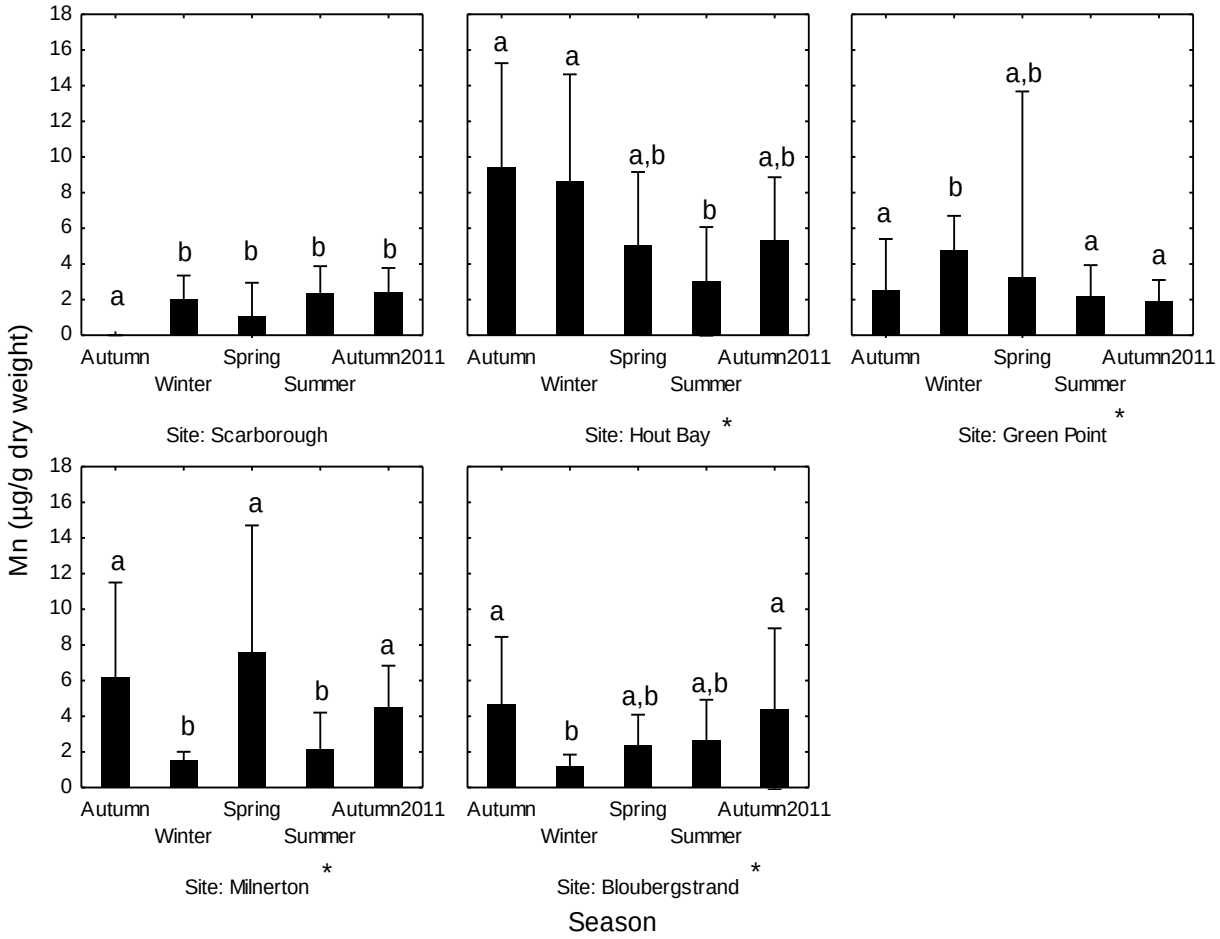

Figure 4-2. Mean Mn concentrations (µg/g dry weight) (± SE) (n=8) measured in *M. galloprovincialis* grouped per season and categorized per site from autumn 2010 to autumn 2011. Different letters indicates statistical differences between seasons using the Bonferroni post hoc test ($p<0.05$). * indicates significant differences from Scarborough (control site) using the Dunnet post hoc test ($P<0.05$).

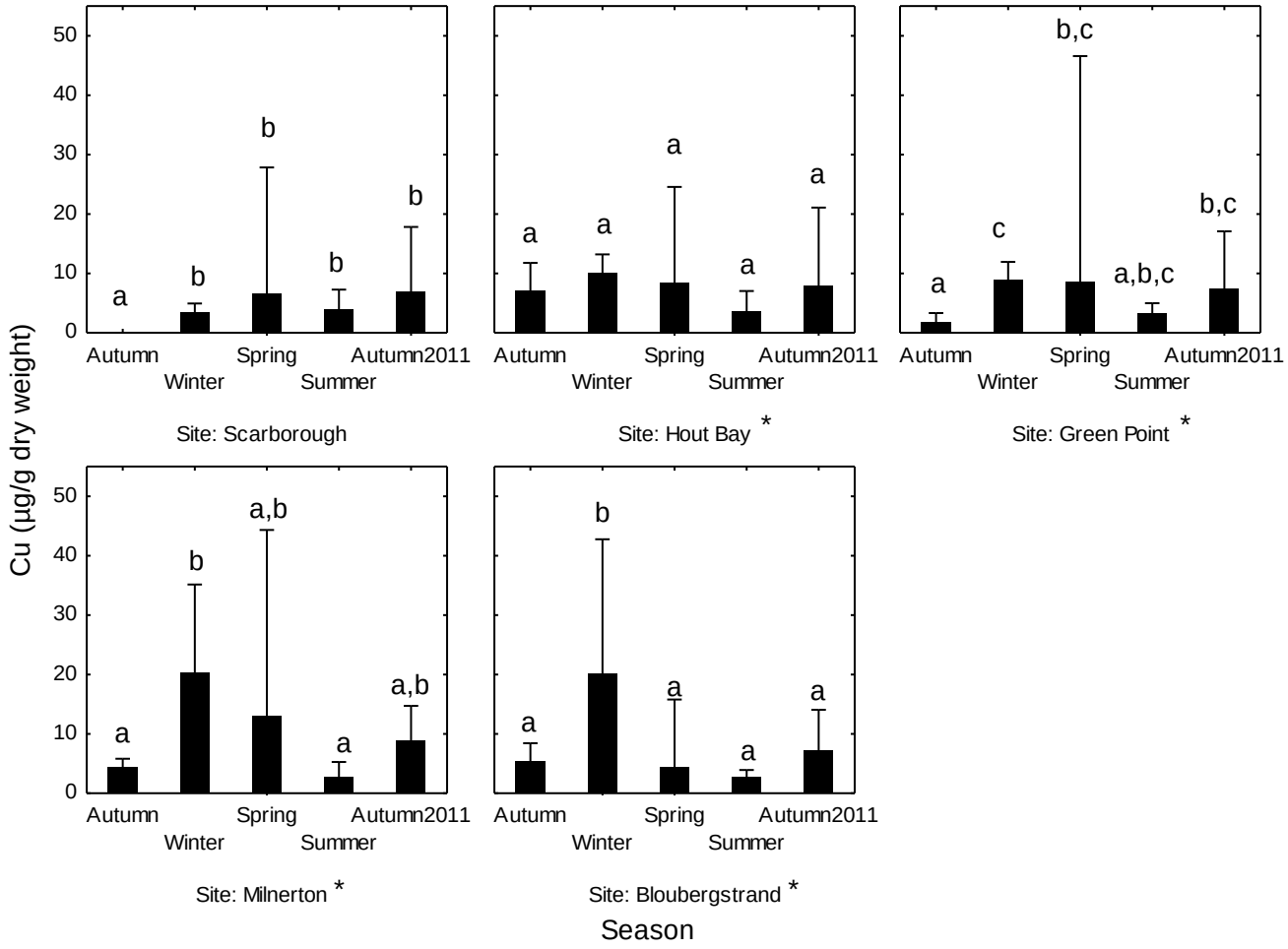

Figure 4-3. Mean Cu concentrations (µg/g dry weight) (± SE) (n=8) measured in *M. galloprovincialis* grouped per season and categorized per site from autumn 2010 to autumn 2011. Different letters indicates statistical differences between seasons using the Bonferroni post hoc test ($p<0.05$). * indicates significant differences from Scarborough (control site) using the Dunnet post hoc test ($P<0.05$).

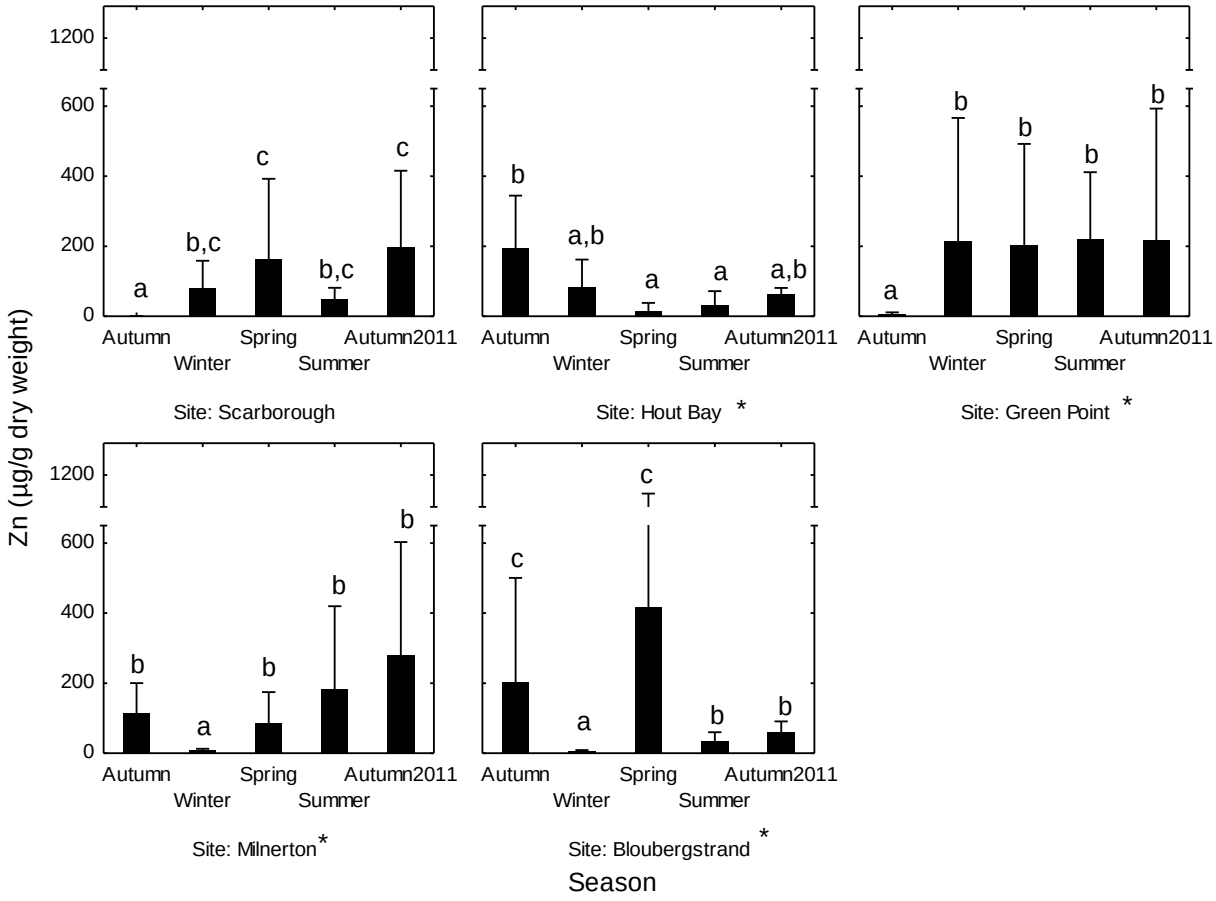

Figure 4-4. Mean Zn concentrations (µg/g dry weight) (± SE) (n=8) measured in *M. galloprovincialis* grouped per season and categorized per site from autumn 2010 to autumn 2011. Different letters indicates statistical differences between seasons using the Bonferroni post hoc test ($p<0.05$). * indicates significant differences from Scarborough (control site) using the Dunnet post hoc test ($P<0.05$).

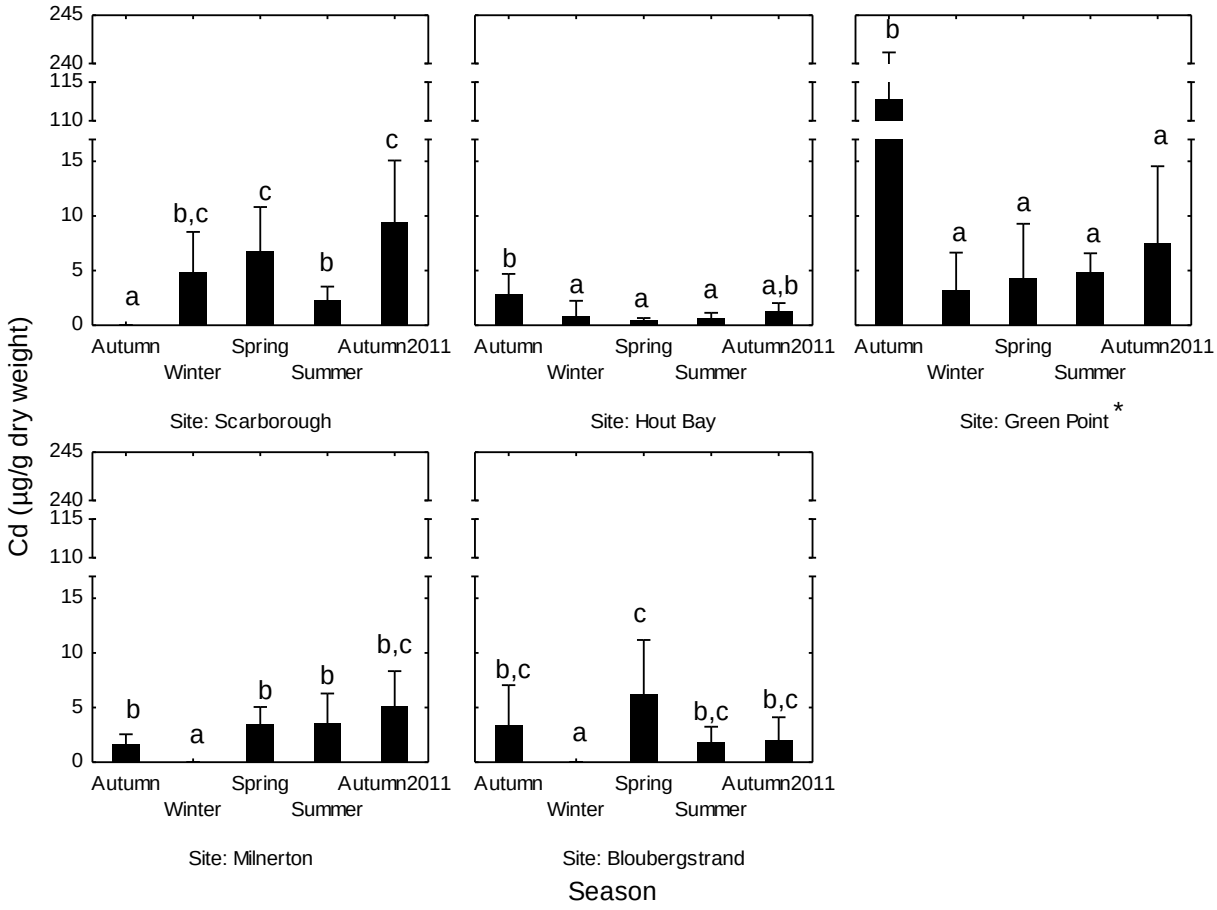

Figure 4-5. Mean Cd concentrations (µg/g dry weight) (± SE) (n=8) measured in *M. galloprovincialis* grouped per season and categorized per site from autumn 2010 to autumn 2011. Different letters indicates statistical differences between seasons using the Bonferroni post hoc test ($p<0.05$). * indicates significant differences from Scarborough (control site) using the Dunnet post hoc test ($P<0.05$).

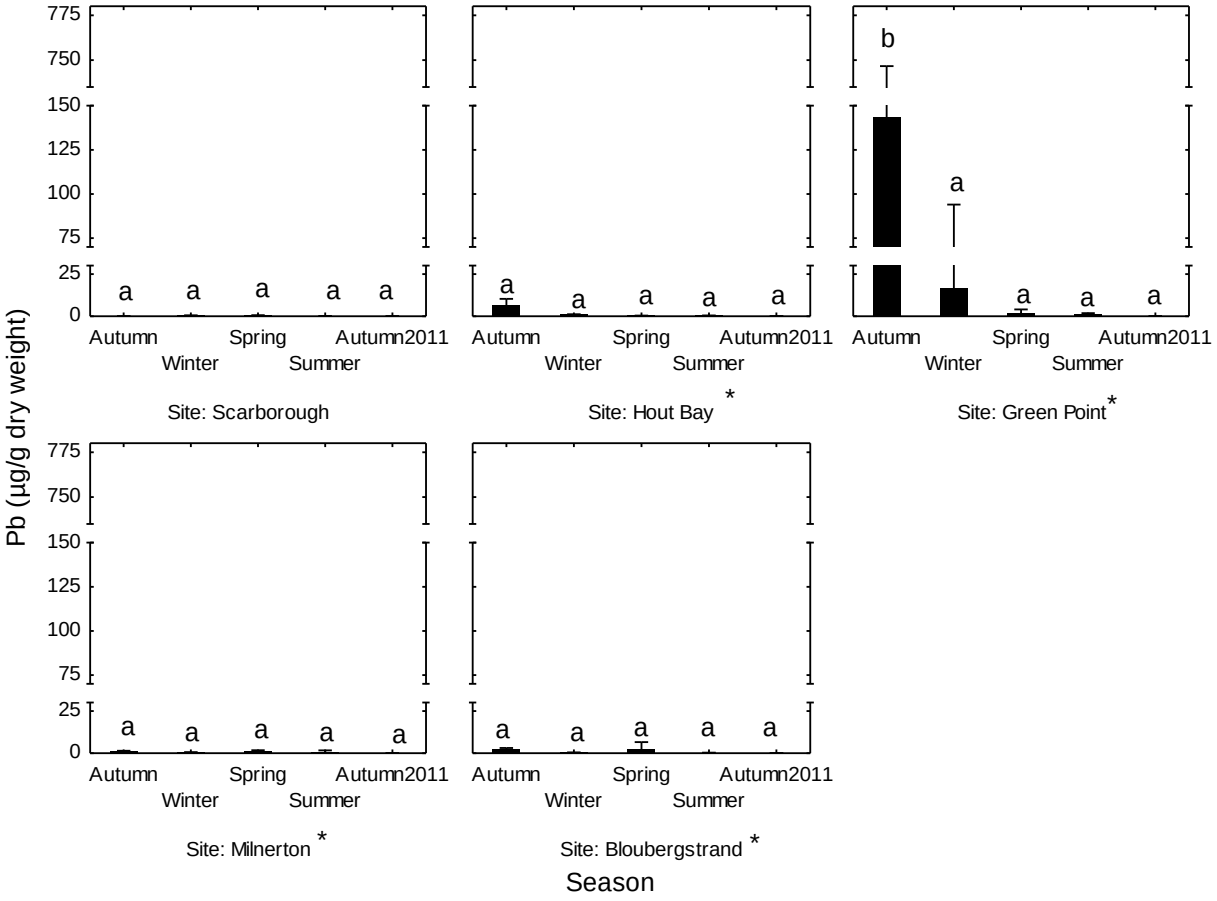

Figure 4-6. Mean Pb concentrations (µg/g dry weight) (± SE) (n=8) measured in *M. galloprovincialis* grouped per season and categorized per site from autumn 2010 to autumn 2011. Different letters indicates statistical differences between seasons using the Bonferroni post hoc test ($p<0.05$). * indicates significant differences from Scarborough (control site) using the Dunnet post hoc test ($P<0.05$).

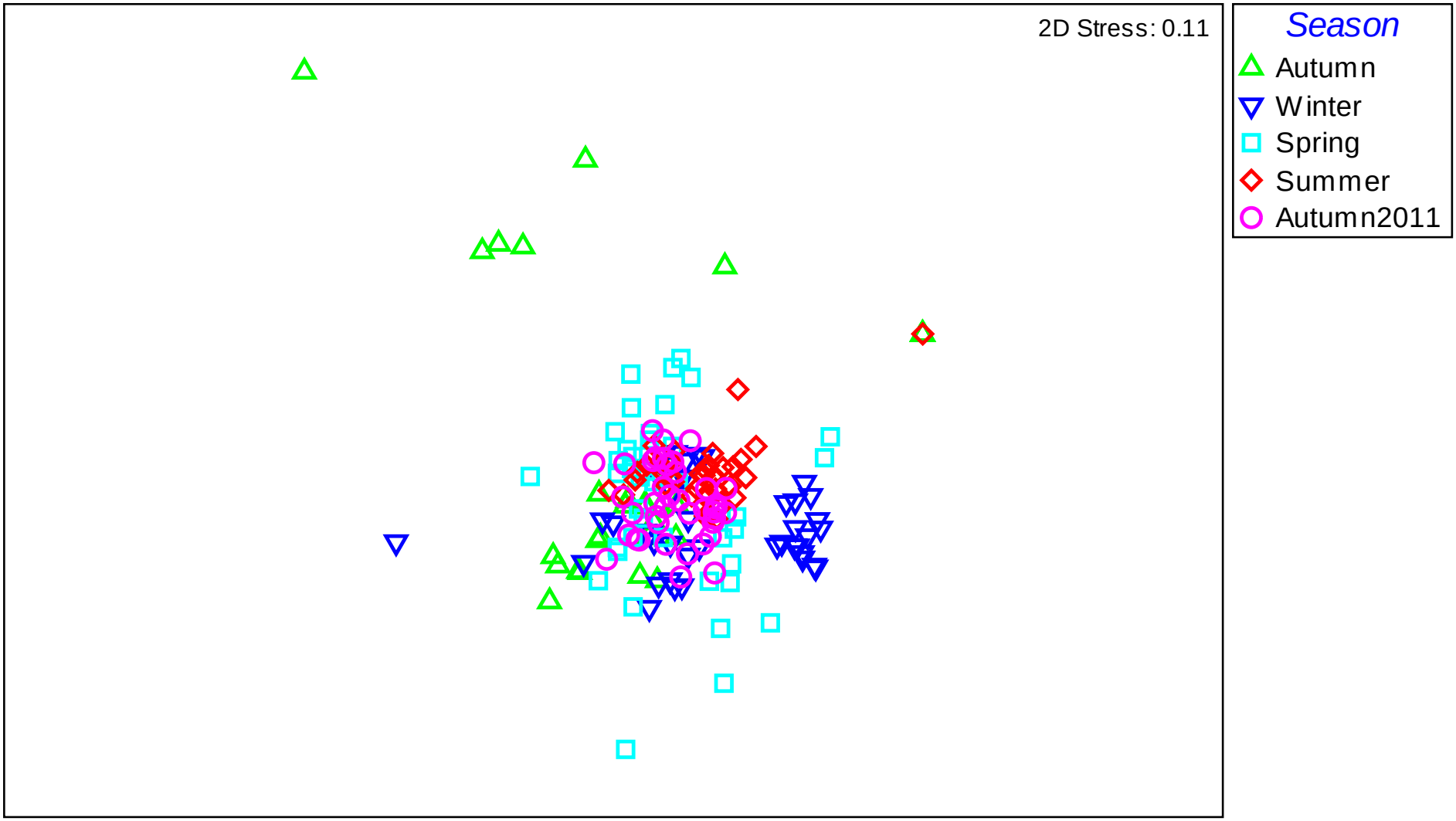

Figure 4-7. MDS ordination of the seasonal data sampled using all metal data. The data were log x+1 transformed and normalised. Euclidean distance was used to plot the samples.

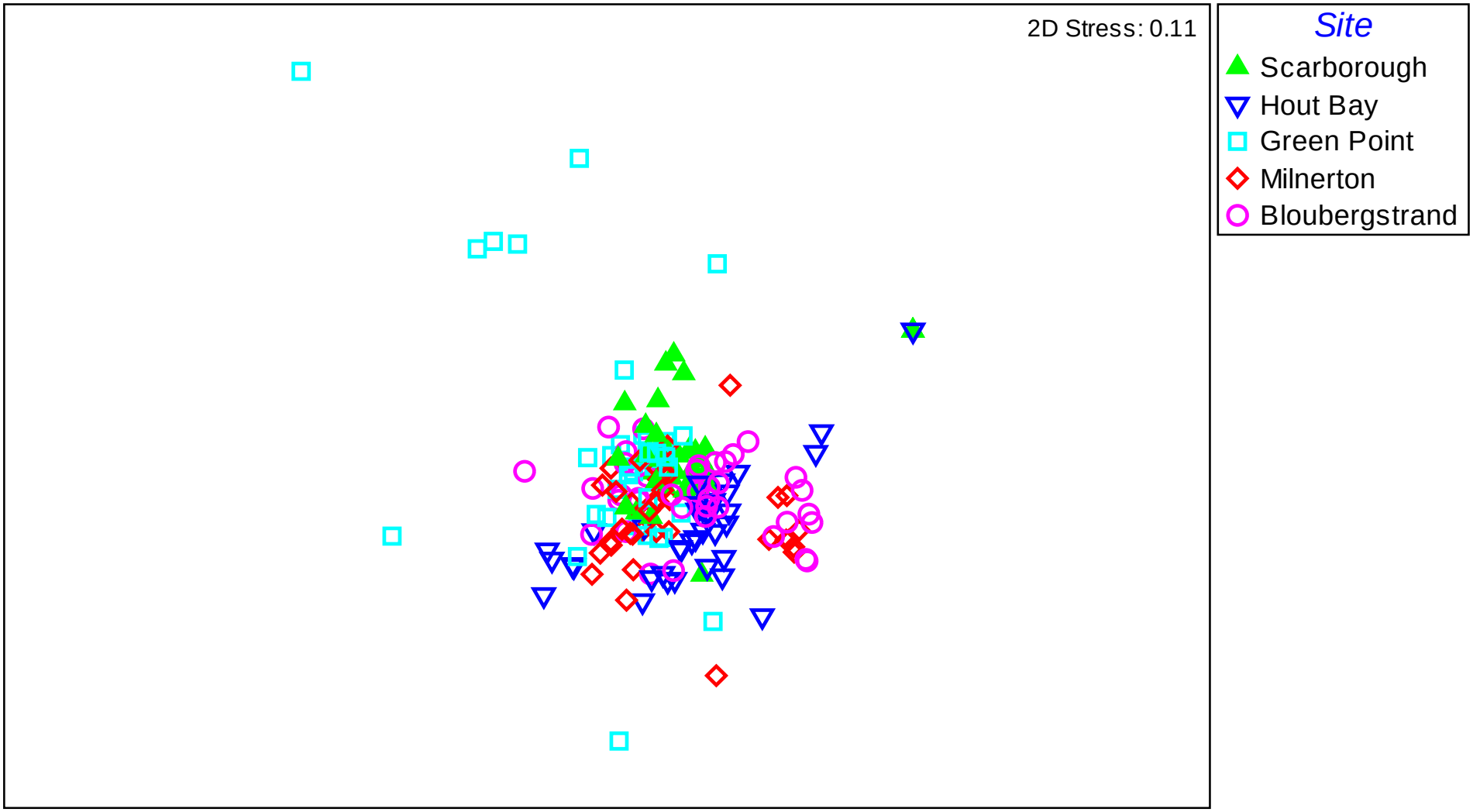

Figure 4-8. MDS ordination of the site data sampled using all metal data. The data were log x+1 transformed and normalised. Euclidean

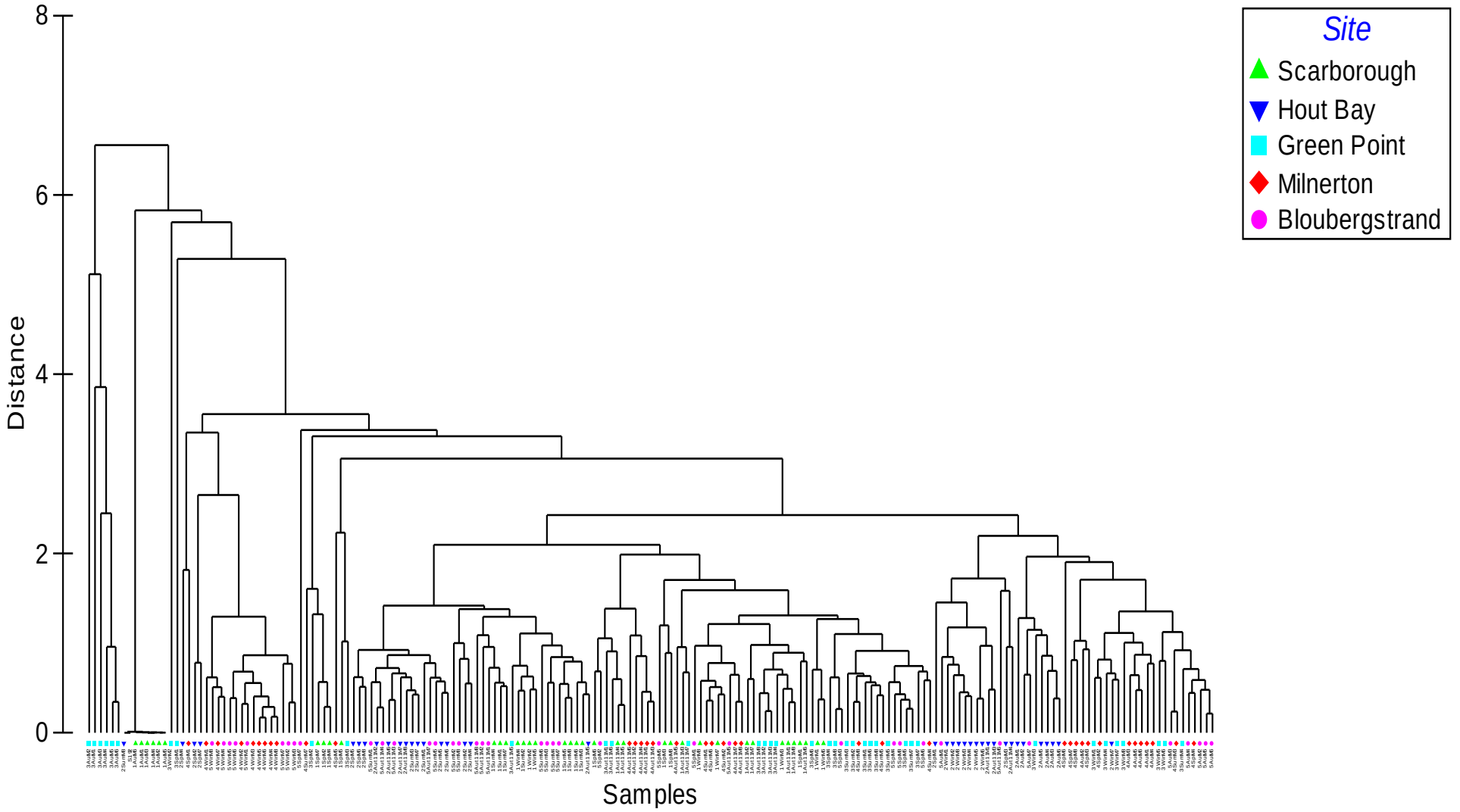

Figure 4-9. Dendogram representing the cluster analysis of metals in mussels for all the sites from autumn 2010 to autumn 2011. The data were log x + 1 transformed and Euclidean distance used for the analysis.

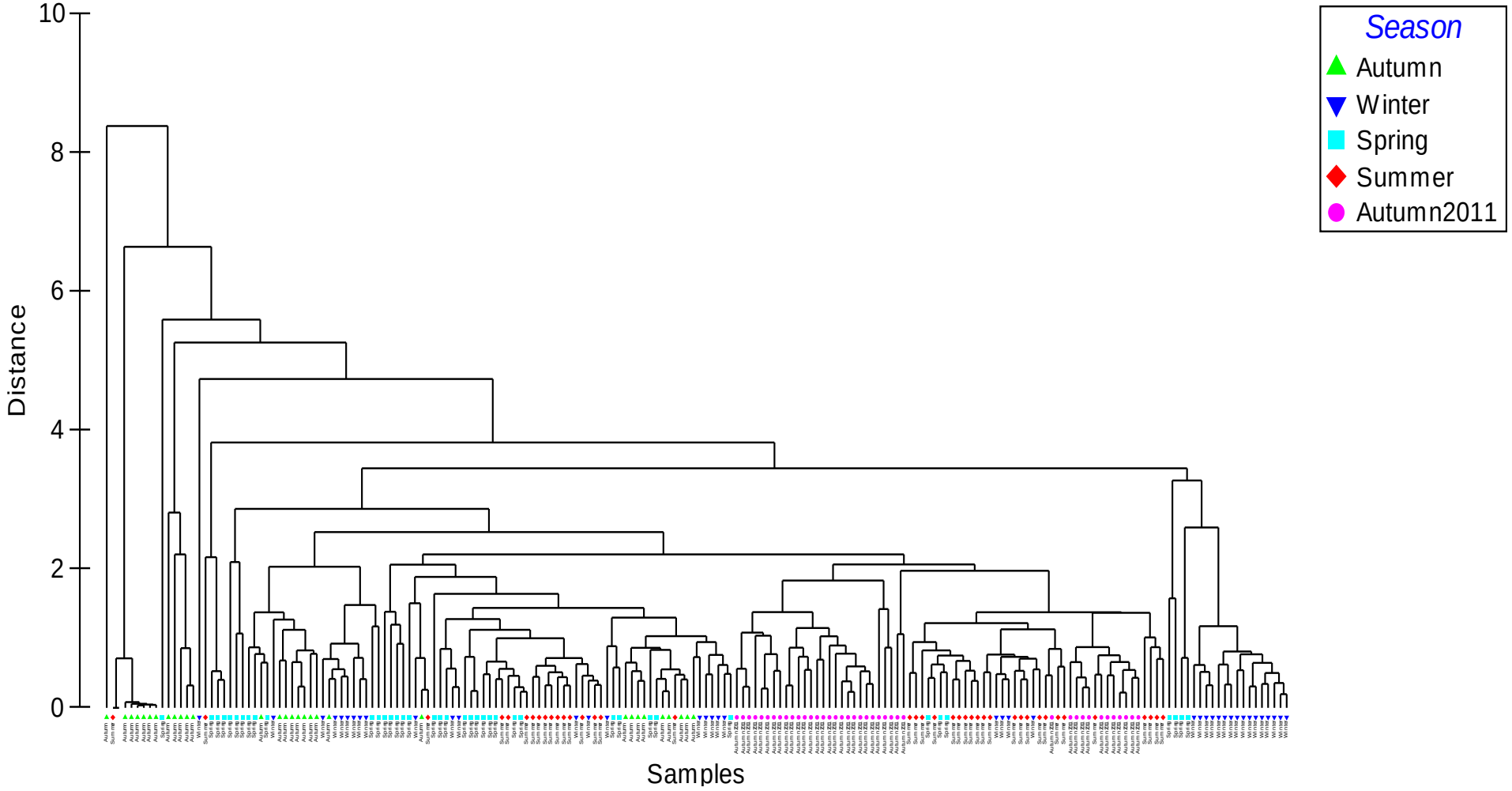

Figure 4-10. Dendogram representing the cluster analysis of metals in mussels for all the seasons from autumn 2010 to autumn 2011. The data were log x + 1 transformed and Euclidean distance used for the analysis.

Chapter 5

ANTIOXIDANT RESPONSES IN *MYTILUS GALLOPROVINCIALIS* EXPOSED TO COPPER UNDER LABORATORY CONDITIONS AND THEIR POTENTIAL AS BIOMARKERS OF METAL EXPOSURE

5.1. Introduction

Metabolic processes involve the systemic breaking down of nutrients that are ingested, into simpler compounds that are able to be absorbed. According to Frei (1994), when in excess, oxidant by-products of normal metabolism can cause damage to DNA, proteins and lipids and this damage is considered a contributor to degenerative diseases. One trade-off to cell damage is that a part of an organism's metabolic resources is devoted to cellular reproduction but at a cost to maintenance. However, the energetic cost of maintenance has an effect on an organism's ability to sustain normal function in somatic cells, including those that defend the cell against oxidants derived from metabolism. It is the accumulation of these oxidants that are detrimental to organism health (Halliwell, 1997; Canesi *et al.,* 1998). As such, the accumulative effects of oxidants have long and short term deleterious consequences to the health of organisms. To combat the deleterious effects of oxidants, organisms use antioxidants to sustain its health (Frei, 1994).

According to Halliwell (1997), antioxidants may be defined as substances that, when present in low concentrations compared to those of the oxidisable substrate, significantly delay, or inhibit, oxidation of that substrate. Antioxidants can be proteins, enzymes or small molecules. According to Niki (2010), there has been significant increase in evidence suggesting the involvement of oxidative stress in the pathogenesis of various disorders and disease. This has attracted much interest in the scientific community and general public about the role of antioxidants in the maintenance of health (mainly human) and prevention of degenerative diseases.

Four endogenous sources of oxidants produced were identified by Frei (1994). Firstly, as a consequence of normal aerobic respiration, mitochondria consume oxygen, reducing in sequential steps to ultimately yield superoxides and hydrogen peroxide (H_2O_2). Secondly, phagocytic cells destroy bacteria or virus-infected cells with an oxidative burst of nitric oxide and hypochlorite. Thirdly, peroxisomes (organelles responsible for degrading fatty acids) produce H_2O_2 as a by-product, which is then degraded by catalase. Fourthly, cytochrome P-450 enzymes constitute one of the primary defence systems against natural toxic chemicals from primary producers and results in the induction of enzymes that prevent acute toxicity effects from foreign chemicals. Three exogenous sources of oxidants were also identified by Frei (1994). Firstly, iron (and copper) salts promote the generation of oxide radicals from peroxides. Secondly, the oxides of nitrogen deplete antioxidant levels and thirdly, diets containing high concentrations of phenolic compounds can generate oxidants by redox cycling.

Whether oxidants are from endogenous or exogenous sources, their ultimate impact is to the detriment of the organism (Duffus, 1980; Bourgoin, 1990; Fent, 2004; Almeida *et al.,* 2007). To counter the effects of oxidants, aerobic organisms have developed defences against oxidative stress by using antioxidants (Torres *et al.,* 2002; Moncheva *et al.,* 2004; Company e*t al.,* 2008). To reduce the negative effects of oxidants, many defence systems have evolved within organisms to limit the impact of oxidative reactions and the damage the reaction may incur (Almeida *et al.,* 2007). Of primary consideration therefore is the development of antioxidant defence systems that includes both enzymatic and non-enzymatic antioxidants (Almeida *et al.,* 2007; Niki, 2010). The endogenous antioxidant defence system includes enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) that act as detoxifying agents of reactive oxygen species (ROS) (Kakkar & Jaffery, 2005; Almeida *et al.,* 2007; Box *et al.,* 2007). Glutathione reductase (GR) catalyses the reduction of oxidised glutathione (GSSG) to reduced glutathione (GSH) and thereby facilitating maintenance of the cellular redox status (Frei, 1994; Box *et al.,* 2007; Niki, 2010).

Antioxidants serve a protective function as the main and first line of defence by suppressing the formation of reactive oxygen and nitrogen species (ROS/RNS)

(Halliwel, 1997; Moncheva *et al.,* 2004; Niki, 2010). This is done by reducing hydrogen peroxide and lipid hydroperoxides to water and lipid hydroxides, respectively, or by sequestering metal ions such as iron and copper. The scavenging antioxidants remove active species rapidly before the active species attack biologically essential molecules. These scavenging antioxidants act as the second line of defence *in vivo*. Superoxide dismutase (SOD) converts superoxide to hydrogen peroxide, while carotenoids scavenge singlet oxygen either physically or chemically (Kennish, 1997; Moncheva *et al.,* 2004; Niki *et al.,* 2010). Many phenolic compounds and aromatic amines act as free radical-scavenging antioxidants. Various enzymes function defensively by repairing damages, clearing the wastes, and reconstituting the lost function, act as the third line defence (Niki *et al.,* 1995; Hwang & Kim, 2007). In addition, the adaptation mechanism functions as the fourth line defence, in which appropriate antioxidants are generated at the right time and transferred to the right position in the right concentration (Niki *et al.,* 1995; Niki, 2010). Furthermore, there is now increasing evidence showing that some antioxidants act as cellular signalling messengers to regulate the level of antioxidant compounds and enzymes (Avery *et al.,* 1996; Kakkar & Jaffery 2005; Almeida *et al..* 2007; Niki, 2010).

In areas contaminated with chemicals, sentinel organisms such as mussels are not able to eliminate toxins and hence accumulate them. Cellular responses to contaminants include the development of mechanisms to protect themselves from toxic effects of increased ROS production (Box *et al.,* 2007). The levels of antioxidants produced are therefore considered potential biomarkers of toxicity of chemicals, hence indicating a contaminant-mediated effect on the organism (Sole *et al.,* 1996; Box *et al.,* 2007).

According to Sole *et al.* (1996), toxicity biomarkers such as malondialdehyde (MDA) have been proposed to reflect toxicity of oxidative stress of an organism exposed to pollution. The level of MDA is used as a marker of oxidation of membrane phospholipids through lipid peroxidation. According to Box *et al.* (2007) an increase in MDA levels in an organism could be related to the degradation of the health status of an environment as a result of poor water quality. This is also evident in marine

species as lipid peroxidation has been shown to be a relevant index of chemical injury induced by toxicant exposure to the species (Avery *et al.,* 1996).

According to Binelli *et al.* (2010), of the many biological responses to pollution that have been recorded in the last 10 years, the investigations based on biomarkers were deemed the most sensitive, reliable and able to represent the earliest signals of environmental disturbance. Since the use of an individual biomarker may not be able to reflect the status of an organism's health and the effects of each pollutant in a mixture (Binelli *et al.,* 2010), the use of a variety of biomarkers (biomarker battery) is considered better suited when determining the biological impact of pollutants (including metals) (Regoli *et al.,* 2004).

Mussels are widely used as sentinel organisms in marine pollution monitoring and are therefore considered ideal to monitor levels of pollution in environments due to their ability to bioaccumulate pollutants (Almeida *et al.,* 2004). Bivalves such as *M. galloprovincialis* are commonly used as bioindicators as these organisms are known to accumulate high levels of trace metals and organic compounds in their tissue (Borkovic *et al.,* 2005). The resultant responses to metal exposure have been shown to be oxidative stress through the formation of ROS and lipid peroxidation (Viarengo *et al.,* 1990). To protect themselves against oxidative damage, mussels have antioxidant defences that reduce the impacts of oxidative stress (de Almeida *et al.,* 2004). As a consequence, ROS generation, oxidation rates and antioxidant status are therefore likely to be related to the ambient environmental status (Wilhelm-Filho *et al.,* 2000).

Antioxidant defence against the damage induced by exposure to toxicants include ascorbate, tocopherol and carotenoids (Frei, 1994). These chemical responses and activities take place via complex biochemical reactions. Irrespective of the nature of the response, there are a number of antioxidant activities that can be used as biomarkers of contamination. Moncheva *et al.* (2004) concluded from a study on antioxidant activity in mussels, that total and free polyphenols can be used as a biomarker of contamination. Other research has indicated that concentrations of metallothioneins (MT) can be used as indicators of the degree of sea pollution (El Ghazi *et al.,* 2003; Domouhtsidou *et al.,* 2004; Rainbow *et al.,* 2004; Santamaria-Fernandez *et al.,* 2004).

Evidence from laboratory experiments have shown that a variety of organisms (from algae to fish) have demonstrated biological responses that were elicited by exposure to dissolved metals (Campbell & Stokes, 1985). These authors suggested that this was as a result of a function of free metal ion concentrations, presented as total dissolved metal concentrations as well as ligands of that metal. As a result, experiments of exposure to metals provide information about the biological uptake and possible reactions to exposure. Such experiments indicating the physiological response to exposure are important as they provide information about the capabilities of metals that enhance the formation of ROS and the resultant oxidative stress.

Cu is an essential metal for many biological systems and it is present in small concentrations in various cells and tissues. Numerous enzymes require Cu as a cofactor for structural and catalytic properties, including cytochrome c oxidase, tyrosinase, dopamine-β-hydroxilase, alcohol dehydrogenase, prolyl and lysyl oxidase (Nath, 1997; Suzuki *et al.,* 2002). These enzymes are important for many biological processes required for growth, development and maintenance. Cu is also a cofactor in Cu/Zn-SOD, which is unique in requiring two essential metals (Cu and Zn) for catalytic function (Company *et al.,* 2008). Several mechanisms have been proposed to explain Cu induced cellular toxicity, including the capacity of free Cu ions to participate in the formation of reactive oxygen species (ROS) (Gaetke and Chow, 2003; Pourahmad *et al*., 2003). The presence of Cu can play an important role in the formation of ROS in biological systems (Halliwell, 1997; Almeida *et al.,* 2007). Hence, the presence of Cu influences the formation of ROS and when the ROS production exceeds the rates of its decomposition by antioxidant defences and repair systems, it leads to oxidative stress (Almeida *et al*., 2007).

Copper (Cu) is known to display a high affinity for thiol groups (Hultberg *et al.,* 2001). According to Pourahmad and O'Brien (2000), *in vitro* oxidation of the cystein thiol group of GSH exposed to Cu resulted in the release of superoxide anions and hydrogen peroxides. Exposure to Cu in oysters (*Crassostrea virginica*) resulted in increased lipid peroxidation (LPO) (Conners & Ringwood, 2000) but at the same time, when exposed to Cu and a GSH inhibitor (buthionone sulfoximine), a lower LPO was reported suggesting a protective role of GSH to Cu toxicity. According to Maria and Bebianno (2011), Cu is a potent oxidant agent in *M. galloprovincialis* as

metallothioneins and LPO level increased after exposure to Cu. These authors suggested that the antioxidant responses to Cu exposure are complex and that the contaminant mixtures interact differently based on target specific tissue and that this may lead to an imbalance in the health status of the mussel.

Cape Town is a major urban centre and has a large reservoir of copper. Van Beers and Graedel (2003) evaluated the uses of Cu in Cape Town and found that the city had approximately 110 million kg of in-use Cu. Although these authors focused on the re-use of Cu by reprocessing it, it can be argued that Cu waste will increase in the future, eventually ending up in the coastal environment. When this happens, the higher concentrations of Cu in the environment could make it bioavailable for uptake, in particular by sentinel organisms such as *M. galloprovincialis*. Internationally, several studies have been carried out on the effects of Cu on antioxidant systems in mussels, especially regarding metal uptake and detoxification (Almeida *et al*., 2007; Company *et al.,* 2008). However, the effects of Cu on the antioxidant system in *M. galloprovincialis* in southern Africa have not been investigated as yet.

Because Cu is an important metal for biological systems to function, has the potential to negatively affect antioxidant systems in mussels and the fact that no such study has been undertaken in the region, the aim of this chapter was to apply a multi-biomarker approach to investigate antioxidant response of *M. galloprovincialis* to Cu exposure. To this end, several biochemical responses of the antioxidant system were assessed in whole soft tissue. Total antioxidant capacity was determined using ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay, antioxidant enzyme activity was determined for catalase (CAT), superoxide dismutase (SOD) while reduced glutathione (GSH) and oxidized glutathione (GSSG) levels. Lipid peroxidation was determined using conjugated dienes (CDs) and thiobarbituric acid reactive substances (TBARS). In addition, the concentration of Cu was measured in *M. galloprovincialis* to determine if there was a correlation between oxidative responses and metal concentrations in whole soft tissue.

5.2. Materials and Methods

5.2.1. Chemicals and Equipment

Sodium di-hydrogen orthophosphate mono hydrate ($NaPO_4$), 2,4,6-Tripyridyl-s-triazine (TPTZ), 1-methyl-2-vinylpyridinium trifluoromethanesulphonate (M2VP), sodium acetate, glacial acetic acid, iron (III) chloride ($FeCl_3$), hydrochloric acid (HCl), ortho-phosphoric acid (H_3PO_4), glycerol, butanol, chloroform, methanol, cyclohexane, ethylenediaminetetra-acetic acid (EDTA) and copper sulphate ($CuSO_4$) were purchased from Merck (South Africa). L-Ascorbic acid (AA), Perchloric acid (PCA), 6-hydroxy-2,5,7,8,-tetramethyl-chroman-2-carboxylic acid (Trolox), 6-hydroxydopamine (6-HD), Diethylenetriaminepentaacetic acid (DETAPAC), fluorescein (Fl), butylated hydroxytoluene (BHT), sodium hydroxide (NaOH), sodium chloride (NaCl), thiobarbituric acid (TBA), 2,2'azobis (2-amidinopropane) dihydrochloride (AAPH), 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), glutathione reductase (GR), oxidized glutathione (GSSG) and reduced glutathione (GSH) were purchased from Sigma-Aldrich Chemical Co. (South Africa). Potassium phosphate (KPO_4) and potassium chloride (KCl) were purchased from Saarchem (South Africa). Hydrogen peroxide (H_2O_2) was purchased from BDH AnalaR®.

The proteins were determined using a Pierce® BCA protein assay kit purchased from Thermo Scientific (South Africa). All reagents were of analytical grade. Reactions for ORAC were measured and read in black 96-well fluorescence microplates (Sigma–Aldrich, South Africa) using a Fluoroskan Ascent analyzer (Thermo Electron Corporation, Finland) and all other reactions were measured and read in clear 96-well microplates (Sigma–Aldrich, South Africa) in a Multiskan spectrophotometer (Thermo Electron Corporation, Finland). All centrifugations were done using a refrigerated bench top centrifuge (Eppendorf 5810R, Eppendorf, Germany).

5.2.2. Experimental design

Mussels *M. galloprovincialis* of similar size (6.62 ± 0.8 cm shell length) were collected from Bloubergstrand, Western Cape, South Africa (refer to Figure 3-1, Chapter 3,

page 3-78) during January 2011 (summer 2010). Although Bloubergstrand was not considered to be a reference site, mean metal concentrations in the sediment at Bloubergstrand (Chapter 3) were not above the Canadian sediment quality guidelines for the marine environment (CCME, 2001). Furthermore, metal concentrations in *M. galloprovincialis* sampled at Bloubergstrand were below the international recommended limits (Chapter 4, Table 4-5). Based on the metal concentration data in the sediment and mussels by that stage of the study, Bloubergstrand was not considered to be polluted, albeit that metal concentrations were higher than Scarborough. A final reason for sampling at Bloubergstrand was that it was closer to the laboratory, and this would have reduced the effects of stress resulting from transporting the animals.

The mussels were sampled at spring 2010 low tide and transported, with site water, to a laboratory at the Cape Town campus of the Cape Peninsula University of Technology (CPUT) where they were allowed to depurate and acclimate for 3 days. The mussels were then separated into triplicate groups of 45 specimens, placed in aerated containers, containing 0.5 L of seawater per mussel. The sea water was acquired from the research aquarium in Sea Point (Department of Agriculture, Forestry and Fisheries, DAFF, South Africa), and passed through a closed filtration system containing 0.10 µm, 0.5 µm and 0.1 µm filters as well as a UV filter before being placed into the aerated containers. After 3 days of acclimation in a temperature controlled room (18 °C), the mussels were fed MicroVert® invertebrate food as per the directions, the water changed one hour later and then dosed with Cu.

The exposure experiment comprised triplicates of a control group (mussels in seawater but not exposed to any metal), a low dosage (40 µg/L of Cu from $CuSO_4$) and a high but sublethal dosage (100 µg/L of Cu from $CuSO_4$) exposure. These values were chosen as they represented the environmental condition for the Cape Peninsula (Chapter 4) for the low dosage group and were considered to be stressed when exposed to 100 µg/L Cu (DWAF, 1995). The first 10 mussels were processed at the start of the experiment (T_0) for both metal and antioxidant analysis. The water was changed every second day and 10 mussels sampled every 7 days (T_0, T_7, T_{14} and T_{21}). Of the 10 mussels sampled, 5 were used for metal analysis and 5 for antioxidant analysis. Mussels to be analysed for antioxidant enzyme activity were

immediately immersed in liquid nitrogen and stored at -80° until the analyses were done. The mussels destined for metal analysis were stored at -20°C until the analyses were done. Water temperature within the system varied between 16-17°C.

5.2.3. Metal analysis

Metal analysis was done according to the method of Odendaal and Reinecke (1999). Frozen mussel samples (n=8) were defrosted, and oven dried for 48 hrs at 60° C in a Memmert drying oven. The soft tissue and shells were weighed and separated before oven drying. The dried tissue samples were weighed and homogenized per individual mussel with a mortar and pestle. Aliquot samples of individual mussels (± 0.2 g) were digested using 10 mL of nitric acid (HNO_3). Samples were then heated to 40 °C in a Grant UBD heating block for one hour, thereafter to 120 °C for 3 hours. The digestates were allowed to cool and then filtered through a Whatman No. 6 filter paper and then through a 0.45 µm membrane micro-filter (Millipore) paper using a needle and syringe. Samples were then placed in plastic centrifuge tubes containing 5 mL digestate and 10 mL distilled water and stored in a refrigerator until further analysis was done. A blank accompanied all samples when analyses of samples were done. The concentrations of manganese (Mn), Iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) was analysed, with 7-8 replicates being done for each metal using the Inductively Coupled Plasma Mass Spectrophotometer (ICP-MS). Concentrations of metals are presented as µg/g dry weight.

The ICP results were calculated as follows:

$$\text{metal} = \left[\frac{\text{ICP reading} - \text{blank}}{\text{mass (g)}}\right] \times \text{dilution factor (20)}$$

All metal concentrations in *M. galloprovincialis* were expressed as µg/g dry weight.

5.2.4. Biochemical Analysis

Prior to chemical analysis, shell length (mm) and whole mussel weight (g) was measured. Thereafter, soft tissues were removed from the shells and weighed. Five

mussels were pooled per group (in triplicate) and freeze dried for 48 hours. Thereafter, the soft tissues of the 5 mussels were combined and split into three aliquots and stored at -20 °C until chemical analysis were done.

Samples were thawed on ice and 0.5 g freeze-dried tissue was added to 5 mL of homogenization buffer and prepared as described by Cheung *et al.,* (2002), with slight modifications. The homogenization buffer contained 50 mM KPO_4, 0.1 M KCl, 0.1 mM EDTA, pH 7.4; with 20% glycerol to protect the enzymes. The samples were homogenized using a glass Potter Elvehjem homogenizer and kept on ice throughout the homogenization process until the homogenates were centrifuged at 12000 x *g* at 4 °C for 30 minutes. The supernatant was retained and kept at -80 °C for subsequent analysis.

5.2.4.1. Antioxidant enzyme activities

5.2.4.1.1. Catalase (CAT)

Catalase activity was determined according to Ellerby and Bredersen (2000). The homogenates were thawed on ice and diluted to (1:5 v:v) homogenate to buffer. To a 96-well plate, an assay mixture containing 170 µL phosphate buffer (50 mM KPO_4 buffer, pH 7.0) and 5 µL of the homogenate sample, in triplicate, was added. Thereafter, 75 µL H_2O_2 stock solution (30% v/v) was added, the solution mixed well and the decrease in absorbance measured at 240 nm (ϵ = 0.00394 $mM^{-1}.cm^{-1}$) in a Multiskan spectrophotometer and the enzyme activity calculated. The results were expressed as mmole/µg protein.

5.2.4.1.2. Superoxide dismutase (SOD)

Superoxide dismutase (SOD) activity was determined according to Ellerby and Bredersen (2000). In essence, the kinetics of the auto-oxidation of 6-HD was monitored at 490 nm and 25 °C for approximately 4 mins. The assay was done by adding, in triplicate, 170 µL DETAPAC solution (0.1 mM) in an SOD assay buffer (50 mM, pH 7.5) to a 96-well plate. Samples were diluted 1:10 (v:v) homogenate to buffer

and the SOD buffer was added to the wells to make up a final volume of 200 µL. A range of sample volumes were assayed (0, 6, 12, and 18 µL) beforehand and the 6 µL volume of sample was added to the wells. To the DETAPAC and sample solution, 15 µL of stock 6-HD (1.6 mM) was added to initiate the reaction, whereafter the combined solution was mixed and the amount of protein used that resulted in 50% inhibition of auto oxidation of the 6-HD was measured spectrophotometrically in a Multiskan reader at 490 nm. The results were expressed as U/mg protein.

5.2.4.1.3. Reduced Glutathione (GSH) and oxidized glutathione (GSSG)

Reduced and oxidized glutathione (GSH:GSSG) levels were determined according to Asensi *et al.* (1999). In this assay glutathione reductase is added and hence both GSH and GSSG measured, which indicates total glutathione presence. For the GSSG determination, the freeze dried mussel tissue were homogenised using 500 mM $NaPO_4$ with 1 mM EDTA (pH 7.5), containing M2VP and centrifuged at 15000 x g for 5 minutes at 4 °C. GSH determination was done on previously homogenised freeze dried mussels without M2VP. This enabled conjugation of GSH for the determination of GSSG. Samples of GSH or GSSG standards (50 µL) were prepared in triplicate and added to 96-microwell plates. To these wells, 50 µL (0.3 mM) DTNB and thereafter 50 µL of GR (1u/50µL) were added. The microwell plates were then mixed and incubated for 5 minutes at 25ºC. To initiate the reaction, 50 µL of 1 mM NADPH was added to each well and the absorbance immediately measured at 412 nm in a Multiskan reader. The change in absorbance in either GSH or GSSG was determined using a linear function. Calibration curves for GSSG and GSH were determined separately and the GSH:GSSG ratios calculated by dividing the difference between GSH and GSSG concentrations by the concentrations of GSSG. The results were expressed as µmol/g.

5.2.4.2. Total antioxidant capacity (TAC): ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity assay (ORAC)

The FRAP assay was carried out as described by Benzie and Strain (1996). The homogenized tissue was thawed on ice and thereafter mixed with 5% PCA (1:1). The PCA mixed sample was centrifuged at 4000 rpm for 5 minutes at 4 °C. Thereafter, 10 µL (in triplicate) of sample and standards were pipetted to microwell plates. The standards comprised various concentrations (0-1000 µM) of AA. Thereafter, 300 µL of FRAP reagent was added to the plates. The FRAP reagent was prepared as follows: 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution, 20 mM $FeCl_3$ solution and distilled water that made up a final volume of 300 µL. The final volume added to the plate was 310 µL. The plate was incubated at 37 ^{0}C for 30 min and read at 593 nm in a Multiskan reader. The results were expressed as µmol Ascorbic Acid Equivalents (AAE) /g.

The ORAC method was performed using a fluorescence spectrophotometer until zero fluorescence occurs. The method of Ou *et al.* (2001) was used on samples that were homogenized as described previously. A 1:10 (5% PCA FRAP homogenate sample: ORAC buffer) diluted sample was used for the ORAC assay. Preparations of the samples were done on ice throughout the whole procedure. The PCA diluted sample was centrifuged at 4000 rpm for 5 minutes at 4 °C. Thereafter, 12 µL (in triplicate) of sample and standards were pipetted to black 96-microwell plates. The standards comprised various concentrations (0-417 µM) of Trolox solutions. Thereafter, 138 µL of fluorescein was added to the plates followed by 50 µL of AAPH, to initiate the reaction, making up a final volume of 200 µL being added to the wells. The solution in the wells was read using a fluorescence plate reader. The fluorescence of fluorescein was recorded every 5 minutes for 2 hours after the addition of AAPH. The ORAC values were calculated using a regression equation ($Y = a + bx + cx^2$) between Trolox concentration (Y) (µM) and the net area under the fluorescence decay curve (x). Data were expressed as micromoles of Trolox equivalents (TE) per milligram of sample (µmol TE/g).

5.2.4.3. Lipid peroxidation: Conjugated dienes (CDs) and thiobarbituric acid reactive substances (TBARS)

Levels of lipid peroxidation were assessed by measurements of conjugated dienes (CDs) and thiobarbituric acid reactive substances (TBARS). The CDs were measured according to Recknagel and Glende (1984). To the freeze-dried samples (50 mg), 1 mL of a chloroform:methanol solution (2:1) was added and kept on ice. Solutions were vortexed and then centrifuged at 10000 rpm for 10 minutes at 4 °C. The top layer was removed and to the bottom organic layer, 500 µL of HCl was added. This solution was then vortexed for 10 seconds and then centrifuged at 10 000 rpm for 3 minutes at 4ºC. From the bottom layer, 100 µL was removed and transferred to a new eppendorf tube and dried under nitrogen gas. To each dried residue eppendorf tube, 1 mL cyclohexane was added. The solution was then placed into micro well plates (in triplicate) and the absorbance measured at 234 nm using a Multiskan reader. The results were expressed as µmol/g.

The TBARS were measured according to Khoschsorur *et al.* (2000) with slight modifications. The homogenized mussel tissue was thawed on ice and 100 µL added to eppendorf tubes. To the samples, 375 µL of H_3PO_4 (0.44 M) and 125 µL TBA (42 mM) was added and the mixture vortexed for 10 seconds and heated in a boiling-water bath for 60 min. The solution was allowed to cool on ice for 2 minutes and then left at room temperature for 5 minutes. To the cooled solution, 500 µL butanol and 50 µL saturated NaCl was added. The sample was vortexed for 10 seconds and then centrifuged at 12 000 rpm for 2 minutes at 4ºC. From this mixture, 150 µL of the supernatant was added to 96 well plates (in triplicate) and the absorbance read at 532 nm in a Multiskan plate reader. The results were expressed as TBARS µmol/µg protein.

5.2.4.4. Total protein concentrations

Protein concentrations in homogenized tissue samples were determined using a commercially-available protein assay kit (Pierce® BCA Protein Assay Kit, Thermo Scientific). Bovine serum albumin (BSA) was used as the protein standard and quantified by measuring absorbance at 595 nm (Bradford, 1976).

5.2.5. Statistical analysis

Data were reported as means (± SE). All calculations and data analysis were done using Statistica v10 (Statsoft). One way ANOVA was used to determine whether there were differences in mean metal concentrations over time and between exposure groups in *M. galloprovincialis*. The data was tested for normality and homogeneity of variance using Kolmogorov-Smirnoff and Levene's tests respectively, prior to post hoc comparisons. When data did not follow these assumptions, they were logarithmically transformed. Post hoc ANOVA analysis were done using the Tukey Honest Significant Difference (HSD) Test to determine statistical significances between groups and over time ($p<0.05$). The use of the test resulted in the determination of significant differences ($p<0.05$) between control, low and high dosage groups as well as significant differences over time (7, 14 and 21 days). Further ANOVA analysis were done using the Dunnet Test to determine statistical significances between groups and time with control groups (no exposure group or at T=0) ($p<0.05$). Non-parametric Spearman rank correlations of assays in *M. galloprovincialis* were done to determine relationships between parameters.

5.3. Results

5.3.1. Metal analysis

Exposure to copper (Cu) in the mussel, *Mytilus galloprovincialis* resulted in metal accumulation in the soft tissue of the organism over the 21 day exposure (Figure 5-1). Within the control group (no exposure to copper) the level of Cu in the tissue of mussels were relatively low and ranged between 1.43 and 2.21 µg/g dry weight (dw).

In the low dosage group, the mussels were exposed to 40 µg/L Cu. Copper accumulated in mussels exposed to low dosages of Cu. At the start of the exposure, the level of Cu in the mussel tissue was 2.83 ± 0.34 µg/g dw and increased 4 fold at the end of the 21 day exposure period to 12.55 ± 1.73 µg/g dw. The mean concentrations of Cu in the low dosage group were 6.81 ± 6.86 µg/g dw. The increase (2.8 fold) in accumulated Cu within mussels was significant after 14 days of exposure from the start of the exposure, ($p<0.05$), with further significant ($p<0.05$)

increases at day 21 (4.4 fold). There was also a significant ($p<0.05$) 3.5 fold increase in Cu in mussels within the low dosage group when compared to the control group. By days 14 and 21 there were significant differences in Cu concentration in *M. galloprovincialis* between the low and high dosage groups. The mussels in the low dosage group had a classic dose-response curve.

Mussels exposed to the high dosage of Cu (100 µg/L) showed a 10 fold increase in Cu accumulated within the tissue. At the start of the exposure, the level of Cu was 2.86 ± 0.76 µg/g dw and by day 21 the level of Cu recorded in the tissue was 28.28 ± 7.3 µg/g dw. The mean concentration of Cu within the high dosage exposure was 15. 42 ± 4.11 µg/g dw. Within the high dosage group, Cu increased 2.66 fold on day 7 (2.86 to 7.64 µg/g dw), then to 22.90 µg/g dw on day 14 (7 fold increase from the control) and then to 28.28 µg/g dw on day 21. The increases at day 14 and 21 were significant ($p<0.05$) when compared to the levels of Cu at the start of the exposure. The high dosage exposure group had significantly higher Cu concentrations (8.1 fold) when compared to the control group as well as from the start of the experiment. The mussels in the high dosage group also had a classic dose-response curve.

5.3.2. Biochemical analyses

Catalase activity did not differ significantly in either group (control, low dosage and high dosage) or over time (Time = 0, 7, 14 and 21 days) ($p>0.05$) (Figure 5-2). Although CAT values increased in the low dosage group by day 7, the results were not significant ($p>0.05$). The CAT activity in the high dosage group increased from day 0 to day 7 but the differences were not significant.

At the start of the exposure, SOD activity was higher in the control and high dosage groups than the low dosage group (Figure 5-3). The differences however were not significant ($p> 0.05$). After 7 and 14 days, SOD activities in the control and high dosage groups were significantly ($p<0.05$) lower than at the start of the exposure. During the entire exposure period, there were no significant differences in SOD in the low and high dosage groups compared with the control groups on those respective days ($p>0.05$).

The GSH level followed the same pattern throughout the exposure to Cu (Figure 5-4) where concentrations increased from the control, through the low, towards the high dosage groups. There were no significant differences between dosage groups at the start of the exposure. By day 7 there were significant ($p<0.05$) differences between low and high dosage groups, as well as between the high dosage and the control group. This situation was the same on days 14 and 21 of the exposure. Furthermore, on day 21 the GSH levels in the low and high dosage groups were significantly ($p<0.05$) higher when compared to that of day 0.

At the start of the exposure, FRAP was higher in the low dosage group than both control and high dosage groups (Figure 5-5) but the differences were not significant. For the remainder of the exposure period, the FRAP activity was constant in all 3 exposure groups. FRAP levels in the control and high dosages on day 14 as well as the high dosage group on day 21 however were significantly ($p<0.05$) higher than at the start of the exposure.

The ORAC activity was highly variable over the entire exposure period and displayed an increase in activity from the control to high dosage group after the start of the exposure (Figure 5-6). At the start of the exposure, the low dosage was significantly higher than the control group ($p<0.05$). On day 7 the high dosage group was significantly higher than the day 7 control group. By day 14 both low and high dosage groups were significantly higher than the control group of day 14 ($p<0.05$). The high dosage group of day 14 was significantly higher from what it was at the start of the exposure. The same pattern as day 14 was observed on day 21. In addition to this, the low dosage group was also significantly higher than the high dosage group by day 21 ($p<0.05$).

The CD levels were similar for all groups and were consistently low for the duration of the exposure period (Fig 5-7). The high dosage group on day 7 was significantly lower than day 0 ($p<0.05$). By day 21, the CD levels slightly increased in the low dosage group, but these were highly variable and not significant.

The TBARS levels were similar throughout the entire exposure period indicating slight increases (not significant, $p>0.05$) from control to high dosage groups (Figure 5-8). The low dosage group on day 7 was significantly higher than the start of the

exposure ($p<0.05$). By day 21, TBARS levels were significantly ($p<0.05$) higher than at of the start of the exposure. The TBARS activity in the high dosage group on day 21 was significantly higher than the control group of day 21 ($p<0.05$).

There were significant positive correlations between Cu, GSH, ORAC, TBARS and group. There were also significant positive correlations detected between Cu, GSH, ORAC, TBARS and day (Table 5-1). When considering exposure, significant positive correlations were recorded between group, Cu, CAT, GSH, ORAC and TBARS. Further significant positive correlations for antioxidants were recorded between GSH and ORAC, FRAP and ORAC, CAT and TBARS. There was only one significant negative correlation recorded, between SOD and FRAP.

5.4. Discussion

Filter-feeding bivalves, such as mussels, are renowned for their ability to accumulate contaminants to high levels (Cheung *et al.,* 2004). Hence, mussels that occur in areas contaminated with xenobiotics may be exposed to oxidative stress due to the effects of oxyradicals (Cheung *et al.,* 2002). It is for this reason that antioxidant responses to anthropogenic influences have been proposed to be used as biomarkers for field-based monitoring programmes (Livingstone, 1998; Cheung *et al.,* 2001). According to Cheung *et al.* (2004), laboratory-based experiments are able to facilitate interpretation of results from field studies and contribute to research of causative factors. The antioxidant defence of marine molluscs have been the subject of numerous studies, focussing on responses to aquatic pollutants (Pannunzio & Storey, 1998). The exposure of *M. galloprovincialis* prevalent in the Western Cape, to Cu, is a first attempt to ascertain if antioxidant responses can be used as biomarkers of metal toxicity.

The possible effect of using mussels from Bloubergstrand as a sampling site was that the mussels could have been stressed by exposure to metals at the time of sampling. However, given that the levels of metals recorded in the present study, and based on historical data, the effect of metals at the sampling site can be considered negligible. Furthermore, if the mussels were stressed by metal exposure, the mussels were collected at the same time, from the same location, and this would have resulted in

all the mussels having the same stress. Hence the mussels would have had the similar stress levels at the start of the experiment, albeit that it was possible that they could have been stressed by factors other than metals as well. This limitation is noted and the results of the study could have been influenced by metal-induced (and other anthropogenic and natural) stress at the start of the experiment. Nevertheless, the data produced typical dose-responses, indicating that the mussel's antioxidant activity responded to the dosages of the experiment.

The literature on the effect of antioxidant enzyme activities in aquatic organisms shows highly variable results, mainly due to the lack of standard and calibrated methods (Almeida *et al.,* 2007; Riva *et al.,* 2010). This is often due to the variety of methods of analyses used to measure antioxidant enzyme activities, ranging from measuring gills, digestive glands and whole organisms. This makes comparison of the results difficult. The present study focussed on antioxidant activities of the whole organism as indications by Osman and Van Noort (2007) found that CAT and GST activities in whole soft tissue in *Dressena polymorpha* were much higher than in the gills (Riva *et al.,* 2010).

Mussel exposure to inorganic copper resulted in the changes to antioxidant activity in this study (Fig 5.1 – Fig 5.8). The results indicated that *M. galloprovincialis* exposed to high dosages of Cu, resulted in the accumulation of Cu in its tissue. By day 21 of the exposure a 10 fold increase in Cu concentration in *M. galloprovincialis* was recorded. This result was higher than that recorded in other studies (Tevisan, 2011) where 3-5 fold increases were reported. Company *et al.* (2008) recorded a 2 to 3 fold increase in Cu accumulation in mussels. However, Wu and Wang (2010) reported a 5 fold increase in Cu in mussels dosed with 50 µg/L Cu. These authors noted that the Cu exposure to mussels could induce metabolic disturbances in mussels. According to Canesi *et al.* (1999), submicromolar concentrations of Cu can affect glutathione and digestive glands in *M. galloprovincialis* after one day of exposure, but was followed by a tendency to recover at longer exposure times. Previous studies on mussels exposed to Cu indicated that exposure to high dosages with the resultant accumulation of Cu in the tissue, resulted in oxidative stress conditions being prevalent (Viarengo, 1990). The effect of high concentrations of Cu was that antioxidative stress became evident in impaired oxidant defence systems (Trevisan

et al., 2011) and an increase in the generation of ROS that leads to lipid peroxidation and DNA damage (Company *et al.,* 2008). These effects were observed by day 21 in low and high Cu dosage groups of this study (Fig 5-4, 5-6 and 5-8) with respect to oxidative lipid damage (measured as TBARS).

The CAT enzyme catalyses the transformation of reactive oxygen compounds, *i.e.*, hydrogen peroxide to water (Cheung *et al.,* 2004). Hence CAT is considered to be an important and sensitive biomarker of oxidative stress, even better than SOD, indicating biological effects on the redox status of marine organisms (Regoli *et al.,* 2002). Riva *et al.* (2010) exposed mussels to environmental concentrations of 4-nonylphenol (4-NP). The results of that study showed no significant differences between the higher dosage groups (1, 10 and 100 µg/L). The lack of significant results were also evident in a study by Matazzo and Marin (2004) who exposed mussels (*Tapes phippinarium*) to 25, 50, 100 and 200 µg/L of 4-NP, and reported no significant differences in CAT activity between the various dosage groups. It was hence argued by Riva *et al.* (2010) that mussels might have high tolerance to antioxidant stress levels due to the presence of haemocytes rich in lysosomal hydrolases, a very efficient lysosomal system and/or an increased production of esterase enzymes after the exposure to toxic substances. Maria and Bebianno (2011) reported that CAT activity was only enhanced at lower Cu concentrations (5 µg/L). The results of that study are supported in the present study, as evident from CAT activity, in particular the responses in the low dosage group on day 21 (albeit that the results were not significant). Dafre *et al.* (2004) reported no CAT activity in mussels exposed to Pb. These authors reported that more studies were needed to investigate and better identify the responses elicited to pollutants (Dafre *et al.,* 2004). The higher CAT activity on day 14 in the low and high dosage groups could be evidence of enzyme activity due to the exposure to Cu. It is possible that the mussels initially responded to the stress, but thereafter was unable to reduce the effects of the high Cu exposure by using CAT, as evident by the higher variability on day 21.

In the present study, SOD activity decreased after exposure to Cu, with significantly lower activities recorded in the control and high dosage groups on days 7 and 14. Thereafter, the SOD activity increased by day 21, but the results were not significant. Copper is an essential element and a co-factor for cytosolic SOD activity, and its

deficiency will impair catalytic activity (Maria & Bebianno, 2011). The results of this study are similar to others (Maria & Bebianno, 2011) who suggested that reduced SOD activity may be due to either increased ROS, degenerating SOD activity which in turn resulted in fewer active isoforms (Monari *et al.,* 2007) or lower Cu presence (Damiens *et al.,* 2006). Both these factors could account for the lower SOD activities in control and high dosage groups on days 7 and 14. By day 21 the mussel was able to alleviate the stresses caused and this may have resulted in SOD activity returning to normal. Furthermore, a reduction in SOD activity was also observed in *Bathymodious azoricus* exposed to 0.4 µM Cu for 24 hours (Company *et al.,* 2004). The results were considered to have confirmed the presence of antioxidant enzymatic activity, reflecting a physiological adaptation to continuous metal exposure. Maria and Bebianno (2011), in their study postulated that SOD produced cytosolic H_2O_2 that lead to a lower SOD activity and that this reaction was linked to an inhibition effect or a negative feedback. This is evident in the CAT activities on day 21 (also, see negative correlation between SOD and CAT in Table 5-1, albeit not significant).

According to Regoli and Principato (1995), similar values between total glutathione and acid-soluble thiols indicated that GSH was the most abundant thiol in *M. galloprovincialis* and that the concentrations of GSSG were negligible in both field and laboratory conditions. Hence, the GSH values reported here are considered indicative of total glutathione concentrations. According to Canesi *et al.* (1999), tissue copper accumulation could possibly result in partial GSH oxidation to GSSG, but according Canesi *et al.* (1998), mussels exposed to the same Cu concentration for 3 days did not result in any change in GSH/GSSG ratios. Canesi *et al.* (1999) noted that Cu-induced decrease in glutathione content was mainly likely due to stimulation of GST activity, which was not measured in this study.

According to Canesi *et al.* (1999), GSH is considered to play a protective role against metal toxicity and it seems that glutathione represents the first line of defence against metals. The effects should however be considered in conjunction with the different routes of metal accumulation, speciation within the cellular environment and sequestration in subcellular compartments which in turn results in different patterns of metal cation interaction with intracellular components (Canesi *et al.,* 1999). In the

current study the significantly higher GSH activities reported in the high dosage group suggests that Cu induces oxidative stress, resulting in higher GSH activity and hence supports the proposal about the protective nature of glutathione noted previously. The results reported in the present study for GSH are similar to those reported by Dafre *et al.* (2004). Those authors reported that GSH increased after exposure to a metal and suggested that the response to oxidative stress and consequent GSH responses was considered a good indicator of oxidative stress in mussels. The result of GSH increase in this study therefore supports the proposal that increased levels of GSH be a good indicator of oxidative stress.

Rajalakshmi and Mohandas (2005) reported that during the initial period of exposure to Cu, cell defence mechanisms may be active and enzymes produced to combat metal stress. The postulation is supported for the control and low dosage groups where low enzyme activity was observed. However, given the higher enzyme activity in the high dosage group, it is suggested that the mussels were not able to alleviate the stress induced by the high Cu dosage and the influx of Cu further impeded elimination and detoxification of Cu (Almeida *et al.*, 2007). These results are particularly evident from the GSH activity of the low and high dosage groups and the higher Cu concentrations in *M. galloprovincialis* by the end of the study period.

Both FRAP and ORAC are considered ideal methods to measure total antioxidant capacity (Niki, 2010). The methods however do not distinguish between reactivity and concentration and are considered semi-quantitative. The FRAP assay measures the ferric reducing ability of a sample and is different from the ORAC assay because there are no free radicals or oxidants applied in the assay (Cao & Prior, 1998). The results of this study show a weak but significant relationship between FRAP and ORAC ($r=0.576$, $p<0.05$). This result shows the importance of using more than one method to measure the antioxidant capacity of samples. These findings are similar to that of Cao and Prior (1998) who compared different analytical methods to measure *in vivo* total antioxidant capacity and found a weak but significant correlation between ORAC and FRA serum ($r = 0.349$, $p<0.05$).

The antioxidant activity from the FRAP assay did not display any significant variation during the first 7 days of exposure to Cu. By day 14, both the control and high dosage had significant differences from the start of the exposure. The significantly

higher FRAP levels for the control group suggests that factors other than Cu dosage could have been responsible for oxidative stress, including insufficient Cu concentration in the water of the control group and stress due to general handling (Snyman *et al*., 2000). The FRAP activity for the low dosage group remained constant throughout the study period and could be an indication that at that concentration, the Cu was beneficial for the mussels to facilitate biological system functioning. The significantly higher values for the high dosage groups suggest that Cu accumulation was responsible for the oxidative stress. It was possible that by day 21 the mussels in the high dosage group had not developed mechanisms to deal with the stress caused by higher exposures to Cu and this is evident in the lower antioxidant capacity (albeit not significant). Namiesnik *et al.* (2008) conducted a study that measured the antioxidant activity and found that mussels exposed to polluted sites recorded higher antioxidant activity in the polluted sites. They further reported a positive correlation between antioxidant activity and polycyclic aromatic hydrocarbons. These results further support the proposal that the mussels were stressed by day 14 and it was possible that by day 21 no longer had antioxidant capacity to deal with the Cu-induced stress.

According to Cao and Prior (1998), the ORAC assay is a method that uses inhibition whereby a sample is added to a free radical-generating system and the free radical action is measured. The ORAC assay uses AAPH as a free radical, and because of this, it measures the capacity of an antioxidant to directly quench free radicals. The result of the present investigation indicates that ORAC differed significantly between groups of respective stages of exposure to Cu, suggesting that mussels in those groups were suffering from oxidative stress. The higher ORAC values hence provide an indication of lower protection afforded in the cellular environment against the potential toxicity of Cu. The data show that, despite antioxidant activity taking place, the higher Cu exposure resulted in an inability to counteract the toxicity of Cu, making the organism more susceptible to oxidative stress (Regoli, 2000).

Two methods were used to evaluate lipid peroxidation damage over the course of the 21 exposure period (Figs 5.7 - 5.8). Each of the methods measured damage at a different stage of the lipid peroxidation process. Conjugated dienes (CDs) represent the initial product of radical attack, a rearrangement of the double bonds in

unsaturated fatty acids (Pannunzio & Storey, 1998). The free electron on a CD can react with oxygen to form a lipid peroxyl radical which further removes a hydrogen ion from a lipid hydroperoxide. The results of CD levels in the present study showed that only the high dosage exposure group differed significantly (lower) on day 7 when compared to the start of the exposure. This suggests that the lack of CDs accumulation in both low and high dosage groups of mussels is indicative of mechanisms that exist to repair or reverse CDs by stabilizing the CD level. By doing so, the CD was not able to react and produce peroxyl radicals. However, by day 21 CD levels in the control and high dosage group decreased to that of the start of the exposure, suggesting that the mussels were no longer able to deal with the stress caused by high Cu exposure and increased Cu body concentrations.

The TBARS assay measures one of the terminal products in the peroxidation consequence of breakdown of lipids, known as malondialdehyde (Pannunzio & Storey, 1998). Pannunzio and Storey (1998) reported that TBARS levels in gastropods showed a dramatic increase in TBARS levels when stressed, but that TBARS levels decreased to control levels when these stresses were removed. Company *et al.* (2004), however, reported that lipid peroxidation decreased significantly when exposed Cu. The results of the present study showed an increase in TBARS levels over the 21 day exposure period with only low (on day 7) and high (by day 21) dosage groups being significantly higher than the start of the exposure experiment. The significant increase is therefore indicative that the tissue has adequate antioxidant defences to deal with the increase in oxygen radical generation associated with the increase in stress from increased Cu body burdens and it was only toward the end of the exposure period that they were no longer able to respond to the stress caused. These results are supported by those of Pannunzio and Storey (1998) who found that the formation of TBARS products accumulated under stressful conditions.

The antioxidative manner in which organisms respond to pollutants is difficult to predict and a high degree of variability has been reported (Regoli, 2000), mainly as a function of the category of chemicals, nature of the exposure, stage of biological and ecological cycle (Livingstone & Goldfarb, 1998; Regoli, 2000; Pytharopoulou *et al.,* 2011; Trevisan, 2011). Although the intent of the present study was to detect mussel

antioxidant response to Cu, and correlate the responses to oxidative stress, interpretation of responses is difficult given the variability previously noted. The responses could have been influenced by other metals in the seawater or arising from the food, as well as variable ambient influences during the exposure experiment. These factors could have influenced the bioaccumulation of Cu during the exposure period, hence alter their responses and could also have influenced the integrity of the sample data (even though every effort was made to minimise the last effect). Nevertheless, the results of the present study showed a clear dose-response to Cu by *M. galloprovincialis* and provided valuable information about the use of antioxidants as biomarkers in these mussels prevalent along the west coast of the Cape Peninsula in Cape Town (refer to chapter 6 for spatial antioxidant responses to oxidative stress). Although Cu bioaccumulated in the mussels, the antioxidant responses were variable and difficult to interpret. Of the antioxidant enzyme activity, SOD and GSH are considered ideal biomarkers. Of the antioxidant capacity biomarkers of FRAP and ORAC, both are considered reliable responses, albeit that the FRAP responses were not significantly different. Lipid peroxidation assays indicated that TBARS are better suited as reliable responses to stress and it is suggested that other types of lipid peroxidation methods be considered. The ecological relevance of biomarkers is higher when, in addition to exposure to pollutants, they are indicative of adverse effects on organisms (Depledge, 1994). The results of this study are indicative of this and can be considered for use as references for field based-studies.

5.5. Conclusion

To come to conclusive decisions about the use of antioxidant responses as biomarkers of toxicity is difficult. Only GSH and ORAC activities were dose dependent during the experiment. Animals react to stress in different ways and these are situation specific. In response to exposure to toxins, antioxidant activity increases and in this way can be used as an indicator of toxicity. When the animals are stressed, the responses peak, then decrease, and this ultimately lead to death if the stress is too intense and/or for too long. It is therefore difficult to interpret at which stage of oxidative stress an organism is, when considering the data from antioxidant

responses. Nevertheless, the antioxidant responses of *M. galloprovincialis* appear to be potentially useful biomarkers, as evident from the responses reported in the present study. The reduced capability of the mussel to neutralise deleterious effects of Cu is evident in high dosage exposures and this would have resulted in an increased susceptibility to oxidative stress disease. Hence the measured parameters represent different aspects of antioxidant responses to Cu and are considered potentially useful biomarkers of metal contamination, particularly if combined with other biomarkers of metal-induced stress such as lysosomal membrane integrity and DNA damage.

Table 5-1. Summary of Spearman rank correlations for antioxidant response. Figures in bold are statistically significant at $p < 0.05$ level.

	Group	Day	Cu (µg/g dry weight)	CAT (mmole/µg protein)	SOD (U/mg)	GSH (µmol/g)	FRAP (µmol/g)	ORAC (µmol TE/g)	CD (mmol/mg protein)	TBARS (µmol/µg protein)
Group	1.000									
Day	0.000	1.000								
Cu (µg/g dry weight)	**0.770**	**0.332**	1.000							
CAT(mmole/µg protein)	0.200	0.297	0.307	1.000						
SOD (U/mg)	0.056	-0.203	-0.085	-0.036	1.000					
GSH (µmol/g)	**0.648**	**0.507**	**0.704**	0.250	0.054	1.000				
FRAP (µmol/g)	0.098	**0.443**	0.246	0.036	**-0.388**	0.300	1.000			
ORAC (µmol TE/g)	**0.557**	**0.411**	**0.682**	0.122	-0.325	**0.637**	**0.576**	1.000		
CD (mmol/mg protein)	-0.164	-0.014	-0.096	0.299	0.315	-0.075	-0.015	-0.212	1.000	
TBARS (µmol/µg protein)	**0.396**	0.249	**0.449**	**0.689**	0.037	**0.480**	-0.052	0.254	0.167	1.000

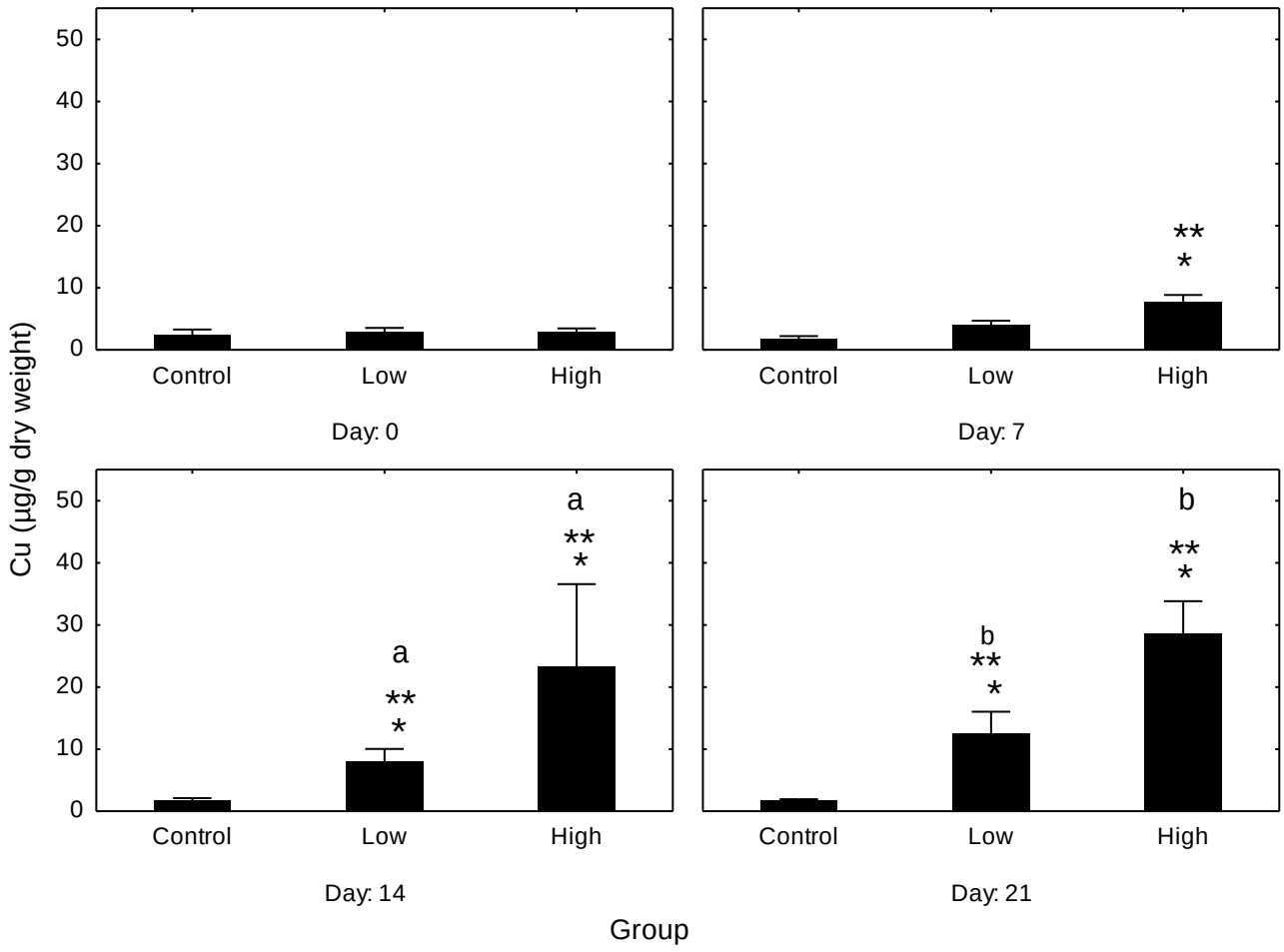

Figure 5-1. Mean (± SE.) copper (Cu) concentrations (µg/g dry weight) in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper. * indicates significant difference of exposure to the control of that group, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test). Similar letters indicate significant difference between exposure groups indicated by $p<0.005$ (one-way ANOVA, Tukey HSD post hoc test). ** indicates significant difference to that of the start of the exposure, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test).

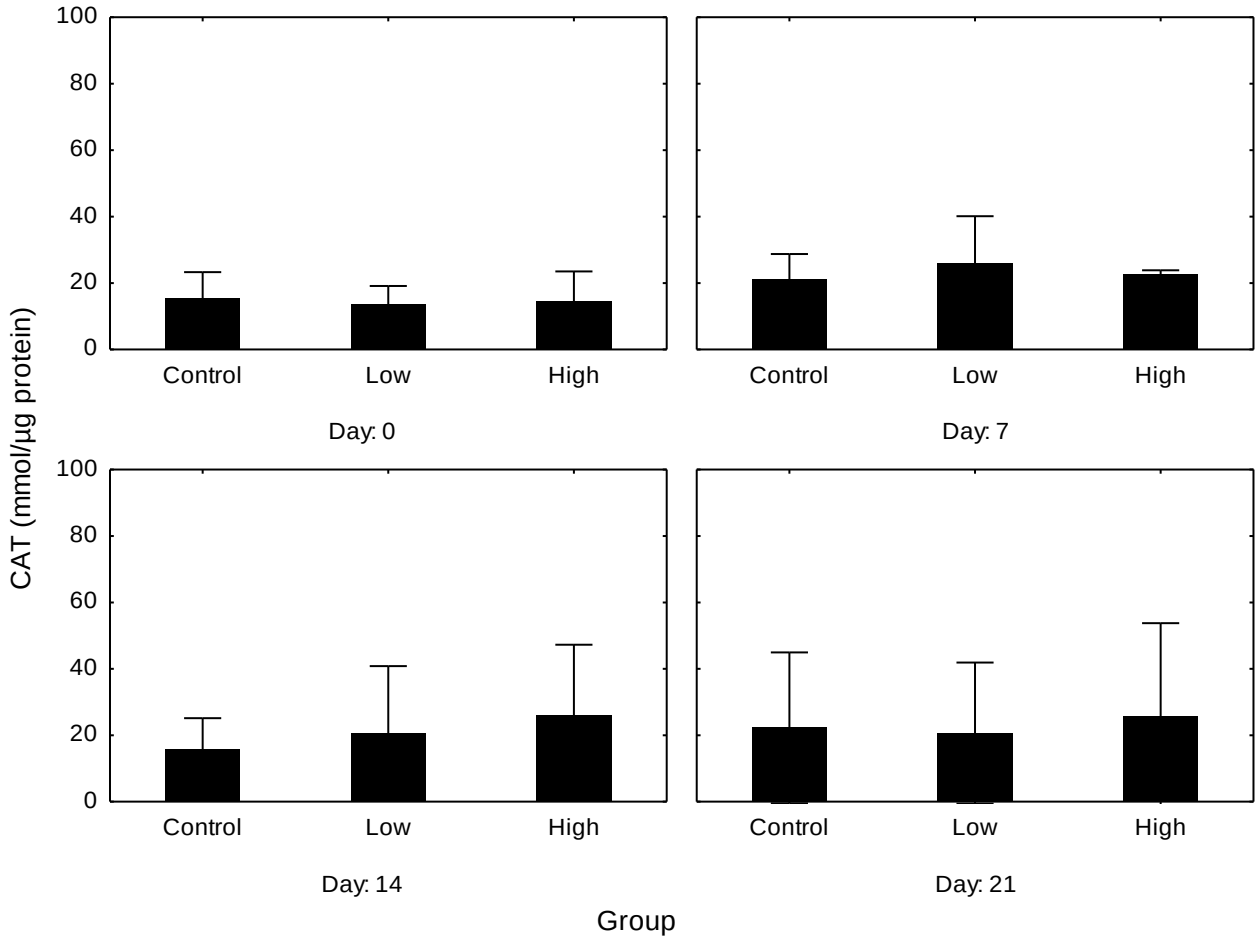

Figure 5-2. Mean (± SE.) catalase (CAT) activity in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 0 µg/L (control), 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper.

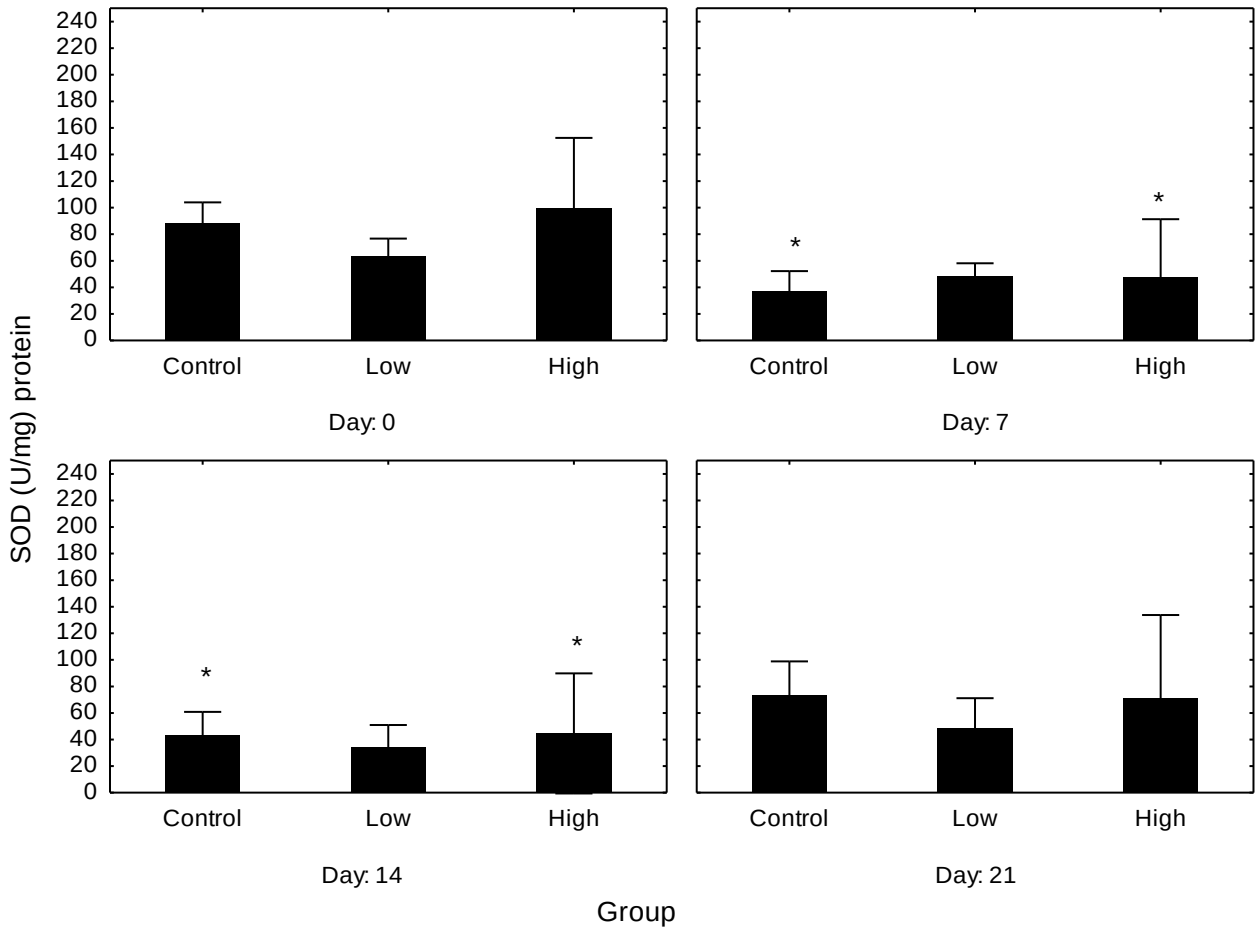

Figure 5-3. Mean (± SE.) SOD activity in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 0 µg/L (control), 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper. * indicates significant difference to that of the start of the exposure, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test).

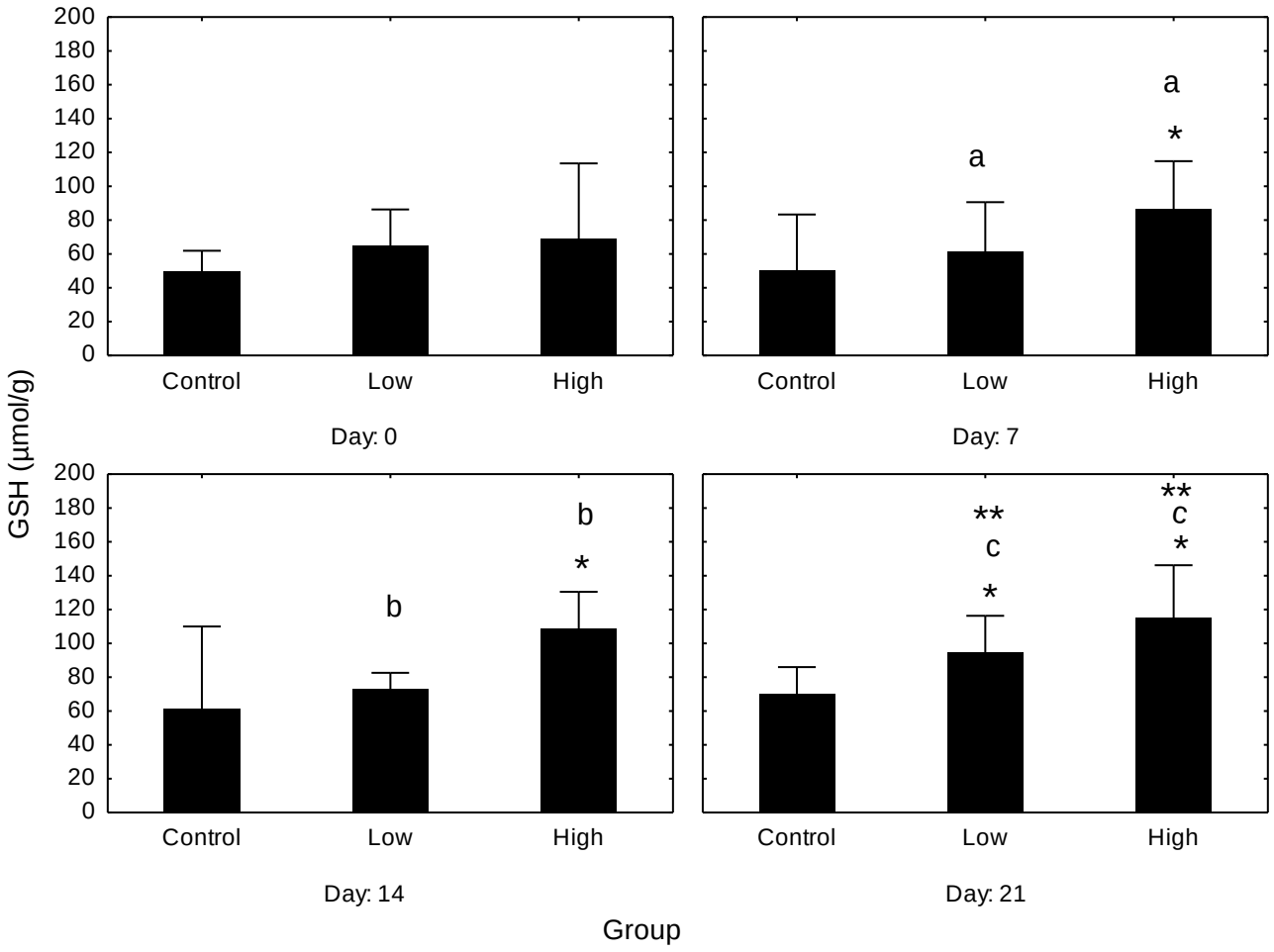

Figure 5-4. Mean (± SE.) GSH activity in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 0 µg/L (control), 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper. * indicates significant difference of exposure to the control of that group, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test). Similar letters indicate significant difference between exposure groups indicated by $p<0.005$ (one-way ANOVA, Tukey HSD post hoc test). ** indicates significant difference to that of the start of the exposure, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test).

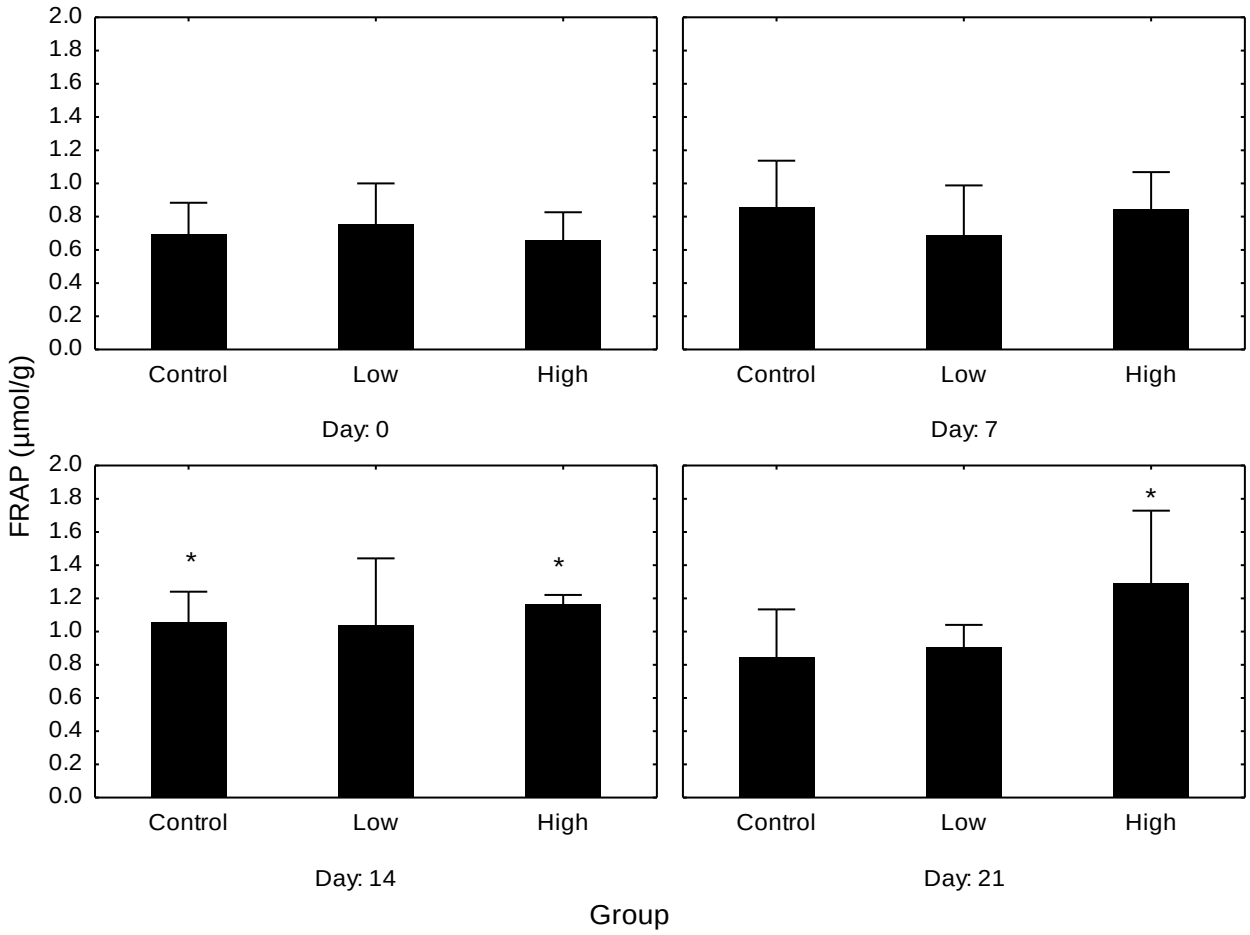

Figure 5-5. Mean (± SE.) FRAP activity in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 0 µg/L (control), 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper. * indicates significant difference to that of the start of the exposure, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test).

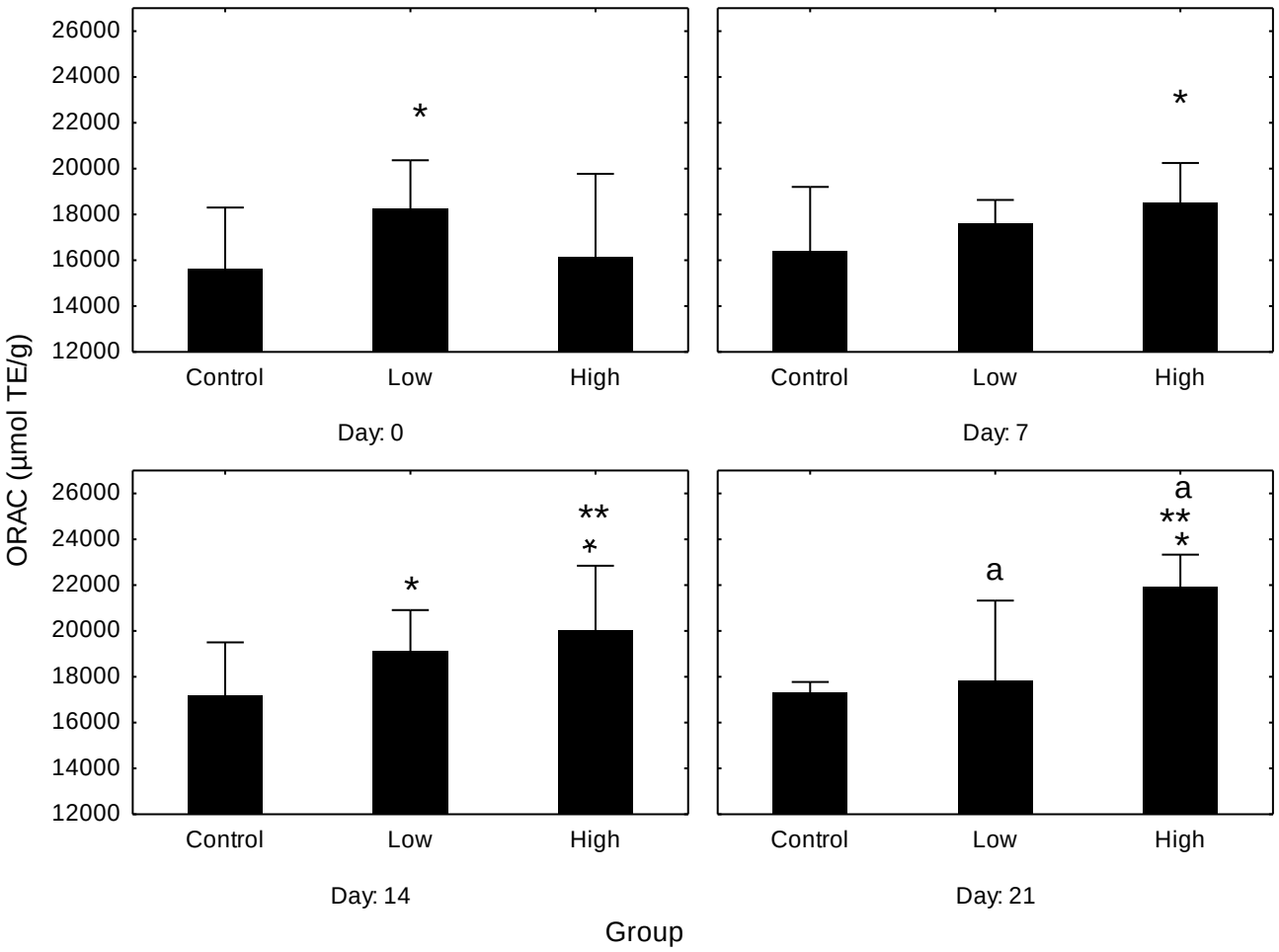

Figure 5-6. Mean (± SE.) ORAC activity in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 0 µg/L (control), 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper. * indicates significant difference of exposure to the control of that group, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test). Similar letters indicate significant difference between exposure groups indicated by $p<0.005$ (one-way ANOVA, Tukey HSD post hoc test). ** indicates significant difference to that of the start of the exposure, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test).

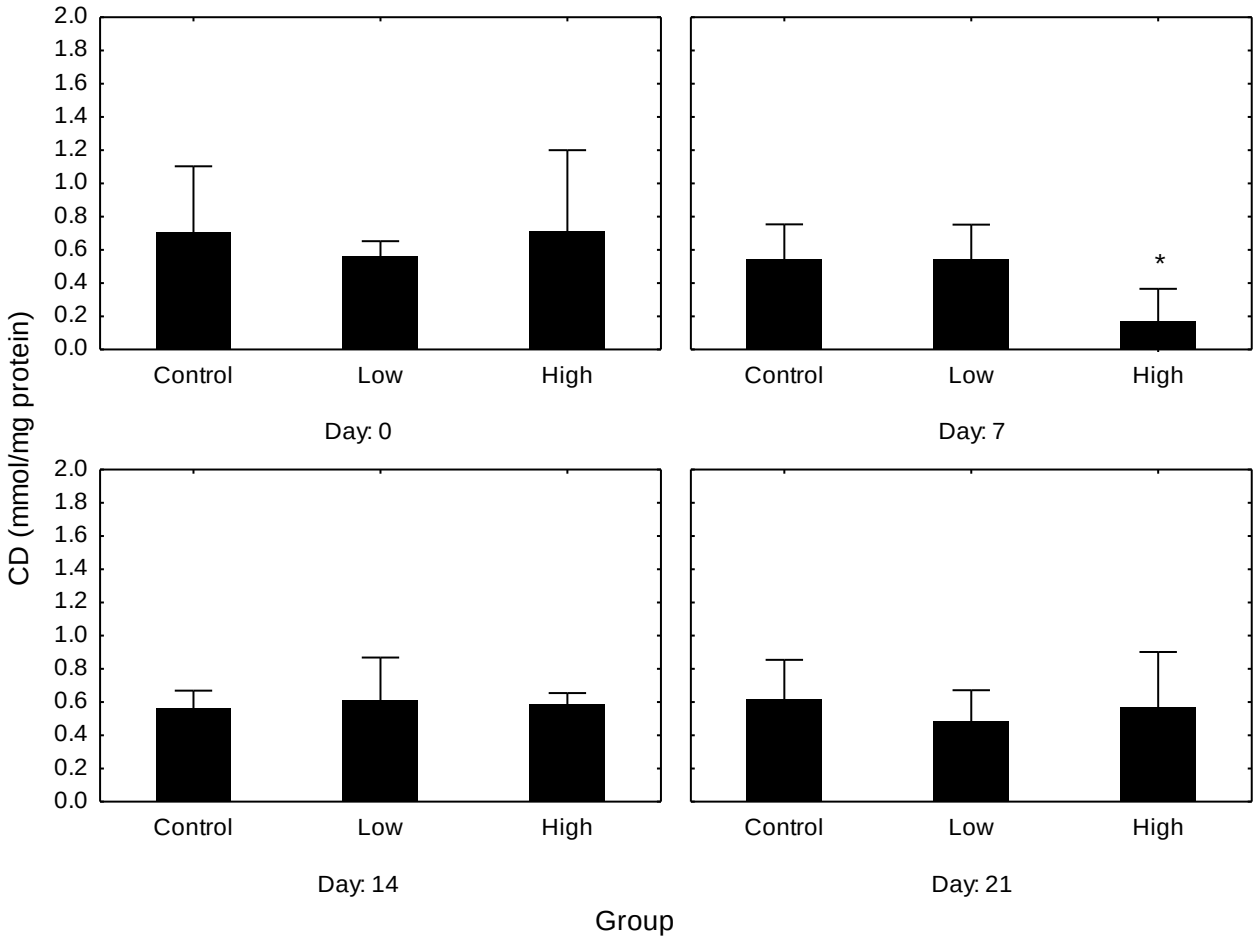

Figure 5-7. Mean (± SE.) CD activity in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 0 µg/L (control), 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper. * indicates significant difference to that of the start of the exposure, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test).

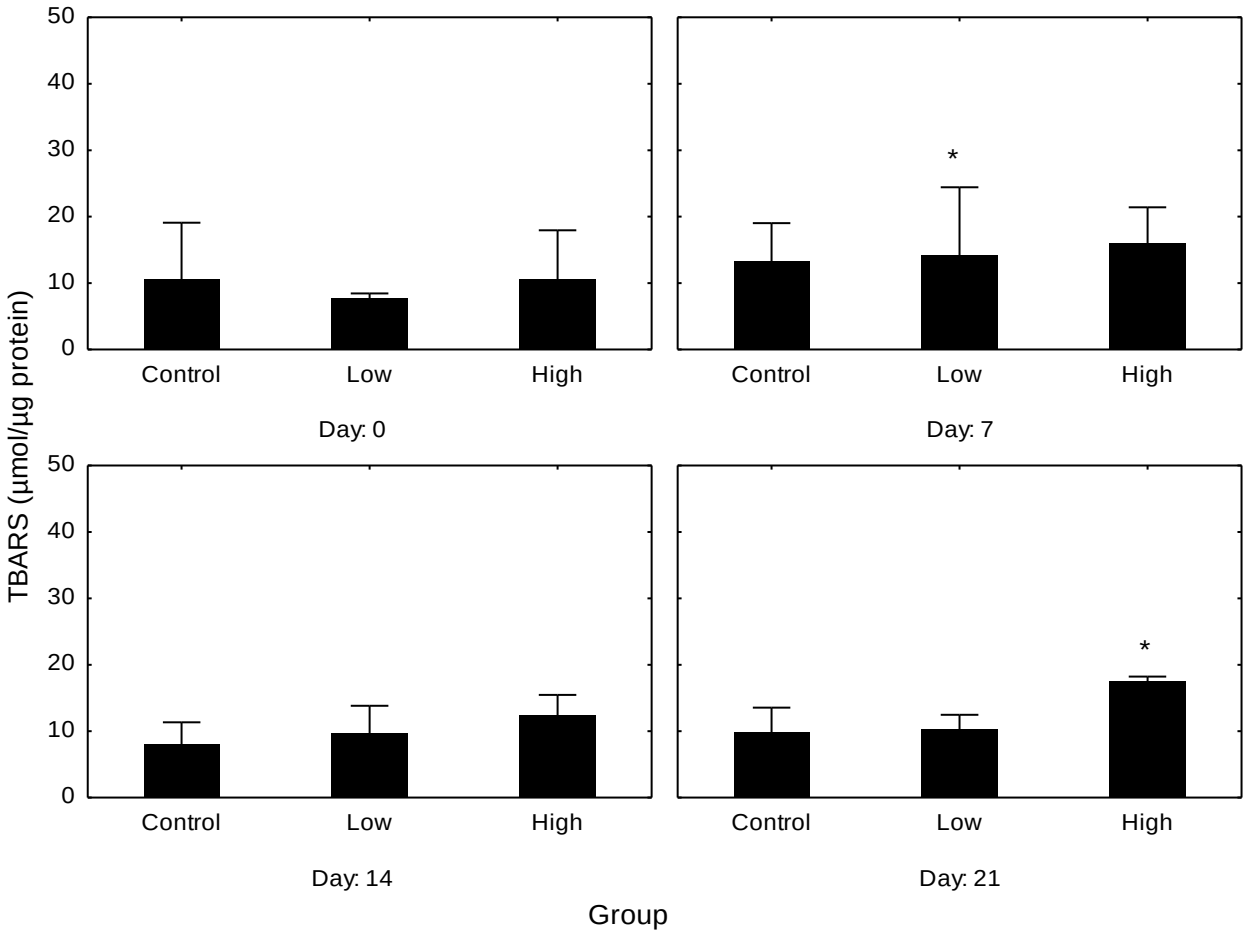

Figure 5-8. Mean (± SE.) TBARS activity in the whole tissue of *Mytilus galloprovincialis* exposed for 7, 14 and 21 days to 0 µg/L (control), 40 µg/L (low dosage) and 100 µg/L (high dosage) of copper. * indicates significant difference to that of the start of the exposure, indicated by $p<0.05$ (one-way ANOVA, Dunnett post hoc test).

Chapter 6

ANTIOXIDANT RESPONSES IN *MYTILUS GALLOPROVINCIALIS* AS BIOMARKERS OF METAL-INDUCED STRESS ALONG THE WEST COAST OF THE CAPE PENINSULA, SOUTH AFRICA

6.1. Introduction

Products of the current era contain an array of metals that may enter the marine environment through discharges from anthropogenic and natural sources. In recent decades, however, development of industrial and urban areas has resulted in more metal contaminated products entering the marine environment, particularly estuaries and coastal areas (Lima *et al*., 2007). The west coast of the Cape Peninsula (in particular Table Bay) is potentially exposed to anthropogenic sources of contamination, including metals, due to the presence of metal by-product industries (petroleum products), residential pollution, and harbours (in Table Bay and Hout Bay). Furthermore, given the high volume of maritime traffic around the Peninsula, the increase in both xenobiotic type and volume could increase exposure to metal-containing contaminants from both the region and other coastal countries.

Monitoring the level and types of pollutants is very important (Gallaway *et al*., 2002; Regoli *et al*., 2002) and various biomarkers have been postulated to monitor the environment (Nasci *et al*., 2002; Riveros, 2002). The choice of biomarkers then needs to be related to the source and type of effect on the environment. Some metals are known to be carcinogenic (arsenic, chromium and nickel) and the effects of these can be modified by exposure to other metals (Kakkar & Jaffery, 2005). Furthermore, toxicity of metals (and other chemicals) is dependent on factors such as absorption, distribution, metabolism and excretion which in turn is influenced by the type of metal, form of metal (species), level of exposure, period of exposure, toxicodynamics and toxicokinetics (Kakkar & Jaffery, 2005). Understanding the dynamics of metal toxicity in the environment is complex and this is further compounded when prevalent in the marine environment. Choosing the ideal

biomarker for the effects of metals on marine organism, population or ecosystem is not easy given the dynamic nature of both the reactivity of metals and the dynamics of the ocean environment.

Antioxidant systems are considered efficient methods to protect organisms against chemical reactive species produced by endogenous metabolism or the change of xenobiotics within the organism (Cossu *et al*., 1997). The reactive species are known as reactive oxygen species (ROS) and are unstable atoms or molecules that try to remove electrons from other molecules to attain more stability and in the process create new radical species that can cause chain oxidations (Fernandez *et al*., 2010). According to Halliwell and Gutteridge (1984), the use of oxidative mechanisms for metabolism is a continuous source of ROS that results in the univalent reduction of oxygen. It was further noted by Winston and Di Giulio (1991) that pollutants (and their metabolites) could be responsible for harmful effects in organisms by their ability to catalyse ROS. If the balance between oxidants and antioxidants is skewed toward oxidants, oxidative stress could occur and ROS could cause tissue damage, disrupt cellular functions, change physicochemical properties of cell membranes and disrupt vital cell and cellular functions that could results in effects on higher levels (Halliwell & Gutteridge, 1984; Manduzio *et al*., 2005; Almeida, *et al*., 2007; Fernendez *et al*., 2010). However, cells have the ability to defend cellular organelles and functions from ROS using antioxidant enzyme systems.

Cells contain antioxidant enzymes that can detoxify ROS, thereby protecting cells from oxidative injury. The three main antioxidant enzymes to protect the cells against ROS are the superoxide dismutases (SOD), which decomposes $O_2^{\bullet-}$ to H_2O_2, catalase (CAT) that decomposes H_2O_2 to molecular oxygen and water, and glutathione peroxidase (GPx) which reduces both H_2O_2 and lipid hydrophiles, associated with glutathione (GSH) oxidation (Hebbel, 1996; Almeida *et al*., 2007; Fernandez *et al*., 2010). Glutathione peroxidase is found in two forms, selenium dependant and selenium independent glutathione peroxidases (SeGP and non-SeGP). The SeGP enzyme contains selenocysteine at the active site and catalyses the reduction of peroxides as well as that of organic hydroperoxides, while the non-SeGP is active only within organic hydroperoxides and is a glutathione transferase (Prohaska, 1980). Glutathione S-Transferases (GST) are considered detoxification

isoenzymes and their substrates could be considered to range from foreign molecules as well as products of metabolism. According to Fernandez *et al*. (2010), GST breaks down the conjugation of GSH to various forms of electrophilic compounds and hence could be considered to play a role as antioxidants (Prohaska, 1980) performing non-enzymatic defences (Almeida *et al*., 2007). Glutathione reductase (GR) is not considered to play a direct role in getting rid of oxygen radicals. However, it is considered to be an essential antioxidant enzyme because it reduces oxidised glutathione (GSSG) and maintains the GSSH/GSH balance and this is vital for cellular homeostasis and the functioning of other enzymes within the cell (Winston & Di Giulio, 1991; Fernandez, 2010).

When cell membranes are negatively affected by ROS, membrane lipids initiate an autocatalytic oxidation process called lipid peroxidation (Almeida, *et al*., 2007). When ROS affect cell membranes, lipids are oxidized, resulting in the formation of lipid hydroperoxides (LOOH) (Almeida *et al*., 2007). Lipid hydroperoxides are also formed via O_2-mediated oxidations (Frankel *et al*., 1979) and via enzymes such as lipoxygenases (Brash, 1999) and cyclooxygenases (Hamberg & Samuelson, 1980). The effects of these reactions are that LOOH in membranes disrupts the normal functioning of cellular metabolism, often resulting in adaptive responses and ultimately death (Girotti, 1998; Almeida, *et al*., 2007). The responses to the effects of LOOH are that phospholipid hydroperoxides can either be detoxified directly by phospholipid hydroperoxide glutathione peroxidase (PHGPx) or by the activities of phospholipase A2 and classical glutathione peroxidase (cGPx) (Ursini *et al*., 1991).

Mussels exposed to metals have been shown to be able to induce oxidative stress via the formation of ROS and lipid peroxidation (Viarengo, 1990; Almeida *et al*., 2004). According to Almeida *et al*. (2004), high concentrations of lipid peroxidation products are correlated with antioxidant enzyme systems (CAT, GPx, GST and SOD) as well as non-enzymatic antioxidants. These authors found that different responses were found in similar experiments, making the interpretation of such results difficult. It has however been accepted that organisms with lowered antioxidant status could be more susceptible to lipid peroxidation, and hence present higher levels of lipid peroxidation (Doyotte *et al*., 1997; Cossu *et al*., 2000).

Antioxidant responses (enzymatic and non-enzymatic) and/or lipid peroxidation can be used as a biomarker to measure oxidative stress in caused by exposure to toxicants in environment and is used to monitor biological-effect responses (ICES, 2007; Fernandez *et al*., 2010). Such monitoring is being seen as an effective means of assessing exposure to, and the effects of pollutants in marine mussels (Regoli & Principato, 1995; Regoli, 1998; Cheung *et al*., 2002; Lionetto *et al*., 2003; Box *et al*., 2007). However, the responsiveness of antioxidants to pollutants is considered difficult to predict due to a high degree of variability in the type of chemicals causing toxicity of ROS, kind of exposure, health of organism as well as phase of life cycle (Livingston, 2001). Nevertheless, both laboratory and field investigations have shown that variations in antioxidants (levels and/or activities) are potential biomarkers of contaminant-mediated biological effect on organisms (Porte *et al*., 1991; Regoli & Principato, 1995; Livingston, 2001).

The results of chapter 5 suggested that antioxidant responses in *M. galloprovincialis* in the Cape Peninsula, Cape Town, could be considered as potential biomarkers of metal-induced stress. Hence, the aim of the present study was to conduct a field validation of the laboratory experiment done by assessing the potential of antioxidant stress responses as a biomarker of stress in the invasive mussel (*M. galloprovincialis*) from five sites along the west coast of the Cape Peninsula, Cape Town (RSA). Numerous biochemical activities and responses of the antioxidant system in the mussel *M. galloprovincialis* were assessed: total antioxidant capacity was determined using ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity assay (ORAC), antioxidant enzyme activity was determined using catalase (CAT) and superoxide dismutase (SOD), while glutathione (GSH) and glutathione disulfide (GSSG) were measured. Lipid peroxidation was determined using conjugated dienes (CD's) and thiobarbituric acid (TBARS) reactive substances. In addition, metals (Mn, Fe, Cu, Zn and Cd) were measured in whole soft tissue of mussels to determine if any correlations between oxidative stress responses and metal levels exist.

6.2. Materials and Methods

6.2.1. Chemicals and Equipment

Refer to Chapter 5 (section 5.2.1) for descriptions of chemicals and equipment.

6.2.2. Sample collection and metal analysis

See Chapter 4 (section 4.2.1) for a description of sample collection. The samples for this study were collected in March 2011. Once collected at the site, the mussels to be used for biochemical analysis were immediately immersed in liquid nitrogen and stored until the analysis was done. The same methods as that of Chapter 4 were applied. Refer to section 4.2.2 of Chapter 4 for a description of methods used for metal analysis.

6.2.3. Biochemical Analysis

Refer to Chapter 5 (section 5.2.4) for a description of the biochemical analysis used.

6.2.4. Statistical analysis

Data were reported as means (± SE). All calculations and data analysis were done using Statistica v10 (Statsoft). One way ANOVA was used to determine whether there were differences in mean metal concentrations in *M. galloprovincialis* between different sites. The data was tested for normality and homogeneity of variance using Shapiro Wilk's and Levene's tests respectively, prior to post hoc comparisons. When data did not follow these assumptions, they were logarithmically transformed. Post hoc ANOVA analysis were done using the Bonferroni test to determine statistical significances between sites ($p<0.05$). The use of the test resulted in the determination of significant differences ($p<0.05$) between different sites. Pearson's correlations were done on metal concentrations and antioxidant activities in *M. galloprovincialis* sampled at the same time to determine relationships between parameters.

To investigate the similarity in antioxidant activity at different sites, PRIMER (Plymouth Routines in Multivariate Ecological Research) V6 was used. PRIMER is a software package that consists of a wide range of univariate, graphical and multivariate routines for analysing arrays of data sourced from community ecology (Clarke & Gorley, 2006). The software has a range of applications including analysis of biological assemblages, but more specifically for this study, application to ecotoxicology by considering antioxidant activity in mussels. The principle application of the software is that data sets are reduced to a triangular matrix representing the (dis) similarity of every pair of sample in terms of their assemblages. Clustering and ordinations techniques can then be applied to display the relationship between samples (Clarke & Gorley, 2006). A multi-dimensional scaling (MDS) ordination of the antioxidant activity measured in *M. galloprovincialis* from all the sites for the study (March 2011) was produced. Data were $\log_{(x+1)}$ transformed and Euclidean distance used to produce a resemblance matrix.

6.3. Results

The results of the present study provided information about antioxidant status at five sites along the west coast of the Cape Peninsula, Cape Town. Of the sites sampled, Scarborough is considered the reference site as it is situated 40 km south of the CBD, away from any industrial and commercial activity. This site is hence considered to be unpolluted, as it is also adjacent to a conservation village and is less than 10 km from the Cape Point Nature Reserve, a national marine park. The low level of metals at that site as evident from data in Chapter 2 (MWP), Chapter 3 (metals in water and sediment) and Chapter 4 (metals in *M. galloprovincialis)* provided support for Scarborough to be considered a reference site.

The CAT levels were significantly ($p<0.05$) the highest in mussels from Milnerton. Scarborough and Green Point (Figure 6.1A), had the second highest concentrations with no significant ($p>0.05$) differences between these sites. Hout Bay and Bloubergstrand had significantly ($p<0.05$) lower CAT activity than the other sampled sites.

Figure 6.1B shows the SOD levels of the five sites sampled. There were generally no significant differences between Scarborough, Green Point and Bloubergstrand. SOD levels in mussels from Milnerton were significantly ($p<0.05$) higher than mussels from the other sites. Hout Bay had the lowest SOD levels ($p<0.05$).

The GSH levels were lowest in Scarborough, increasing toward Milnerton and then decreasing in Bloubergstrand (Figure 6.1C). GSH levels in Milnerton was significantly ($p<0.05$) higher than that of the other sites. The GSH levels measured in mussels from Scarborough were significantly lower than any of the other sites sampled ($p<0.05$).

When considering the antioxidant capacity, only Bloubergstrand had significantly ($p<0.05$) higher FRAP values (Figure 6.2A). When considering the ORAC values, the antioxidant capacity was the highest in mussels collected from the Green Point and Milnerton areas (Fig 6.2B), with the lowest ORAC being recorded in mussels collected from Hout Bay and Bloubergstrand. Scarborough ORAC values were significantly ($p<0.05$) higher than for Hout Bay and Bloubergstrand but significantly ($p<0.05$) lower than that recorded at Green Point and Milnerton.

Lipid peroxidation levels were lowest in mussels harvested from Scarborough and Hout Bay areas (Figure 6.3A). The CD levels were significantly ($p<0.05$) higher in Green Point and Bloubergstrand with Milnerton showing the highest significant ($p<0.05$) CD levels. The TBARS levels showed no significant differences between Scarborough, Hout Bay and Bloubergstrand (Figure 6.3B). Green Point and Milnerton TBARS levels were significantly higher than the other sites with Milnerton having the highest significant ($p<0.05$) levels of all the sites sampled.

The MDS visual presentation (Figs. 6.4-6.7) made it possible to have a visual presentation of the 5 sites sampled with their respective antioxidant status. An analysis of similarity (ANOSIM) test detected significant differences in antioxidant responses for all antioxidant activities ($r = 0.973$, $p<0.01$). An MDS plot of all antioxidant responses revealed distinct differences between sites (Figure 6.4). Although Scarborough, Hout Bay and Green Point were generally grouped, the grouping within the sites was widespread. Antioxidant responses from mussels collected at Milnerton and Bloubergstrand were similar. An ANOSIM of antioxidant

activity revealed similar significant differences between sites in antioxidant activities ($r = 0.904$, $p<0.01$). Here too, the MDS plot indicated sparser groupings at Scarborough, Hout. Bay and Green Point, with closer groupings in Milnerton and Bloubergstrand (Fig 6.5). The ANOSIM of antioxidant capacity revealed that significant differences between sites prevailed ($r = 0.831$, $p< 0.01$). The MDS showed that only Hout Bay data was sparsely distributed with the remaining sites closely grouped (Figure 6.6). An ANOSIM of lipid peroxidation revealed that there were significant differences between sites ($r = 0.959$, $p<0.01$). Bloubergstrand data were spread far apart with the remaining sites being closely grouped (Figure 6.7).

6.4. Discussion

The present study is the first to provide information about the antioxidant status in *M. galloprovincialis* in the Western Cape, South Africa. According to Santovito *et al.* (2005), the presence of organic and metal contaminants in mussels can be possible causes of oxidative stress and could have induced various antioxidant responses. Hence, studies that have demonstrated that antioxidant responses can be induced by oxidative stress, suggest that there could be a significant correlation with these responses to pollutant concentrations (Viarengo *et al*., 1990; Cheung *et al*., 2001; Cheung *et al*., 2002; De Luca-Abbott *et al*., 2005; Jena *et al*., 2009). The results of the present study had also indicated this to be the case. The accumulation of pollutants in bivalves is influenced by a dynamic balance which results in uptake or depuration of these substances and these actions in turn are influenced by a dynamic equilibrium amongst pollutants in sediment, water, food particles and the organisms themselves (Livingstone, 1991).

The results of the present chapter differs considerably with that of Chapter 3, where a pollution load index rating of the various study sites from lowest to highest pollution loads were recorded as follows: Scarborough < Milnerton < Green Point < Bloubergstrand < Hout Bay. The CAT activity in the present study was significantly higher at Scarborough, Green Point and Milnerton whereas for both SOD and GSH, higher activities and levels were at Milnerton. Both antioxidant enzyme activities and GSH levels suggest that Milnerton is a polluted site. According to Jena *et al*. (2009),

CAT, SOD and GSH in mussels from polluted sites showed higher activity and levels when compared to sites that were not considered to be polluted. The high variability of CAT at Scarborough could however be as a result of some other stress other than metal pollutants, for example wave action, desiccation, food availability and even other pollutants not measured (Almeida *et al.,* 2007). Scarborough was the first antioxidant station sampled and it is possible that sampling error could have resulted in the organisms suffering from stress before or while they were being preserved for the antioxidant analysis. Alternatively, a natural factor (higher temperature due to low tide or strong wave action specific to that site) could also have contributed to the higher CAT values recorded at Scarborough (Almeida *et al.,* 2007). CAT are the primary scavengers of H_2O_2 in the cell and the increased CAT activity recorded in Scarborough (compared to the other sites) could indicate stress (anthropogenic or natural) resulted in elevated formation rates of H_2O_2. These results are comparable to others where CAT activity increased due to exposure to metals (Torres *et al*., 2002; Jena *et al*., 2009) and petrochemicals (Verlecar *et al*., 2008).

ANOVA of antioxidant capacity indicated that if FRAP was used as an indicator, Bloubergstrand was considered a polluted site whereas if ORAC was used, Milnerton and Green Point were considered polluted sites. According to Gorinstein *et al*. (2006a; 2006b), antioxidant capacity in *M. galloprovincialis* from polluted sites is significantly higher than mussels from non-polluted sites. If the two antioxidant capacity assays are to be considered together, then Bloubergstrand, Milnerton and Green Point are to be considered polluted sites.

According to Jena *et al*. (2009) mussels with high lipid peroxidation levels are considered to be subjected to pollution stress. If the same criteria were to be used for the present study, mussels in Milnerton are to be considered under high stress if using both CDs and TBARS as criteria. The CD measurement also suggests that Bloubergstrand is subjected to pollution stress. These results then support the finding in Chapter three where it was reported that Bloubergstrand had the second highest pollution load index. The lipid peroxidation results further suggests that Scarborough and Hout Bay are not subjected to pollution stress if the criteria of Jena *et al*. (2009) are to be used. Similar results have been recorded where an increase in tissue pollutants in bivalves was accompanied by an increase in TBARS (Torres *et al*.,

2002; Cheung *et al*., 2004). A possible factor affecting the antioxidant responses is that wild *M. galloprovincialis* were used and hence could have been the cause of the high variability reported here. However, other authors used animals from the wild and found that TBARS in mussels were used to indicate that sites were polluted (Torres *et al*., 2002; Cheung *et al*., 2004; Jenna *et al*., 2009).

The MDS visual presentations showed clear discriminations between stressed and unstressed sites. According to Clarke and Warwick (1994) this could also be an indication of polluted and clean sites. According to Box *et al*. (2007), MDS representations are useful tools to obtain a visual representation of the pollution status of study sites. Box *et al*. (2007) conducted a study on *M. galloprovincialis* in the Balearic Islands using oxidative stress biomarkers and found that the MDS analysis allowed for discriminating impacted sites compared to a reference site. The results of that study suggested that antioxidant enzyme activities in the polluted sites were more similar and homogenous than cleaner sites where higher dispersion of results was evident. These authors accounted for the higher dispersion by stating that it was due to particular abiotic parameters such as temperature, currents whereas the similarity of polluted sites were due to similar compounds such as metals and persistent organic compounds. If these criteria were to be applied to the present study, antioxidant responses showed that Milnerton and Bloubergstrand could be considered polluted, Hout Bay and Green Point mildly polluted and Scarborough, unpolluted (Figure 6.4). The results are the same when considering antioxidant enzyme activities (CAT, SOD and GSH) as criteria to measure polluted and unpolluted sites (Figure 6.5). The MDS of antioxidant capacity suggested that Bloubergstrand, Green Point, Milnerton and Scarborough are polluted and Hout Bay being unpolluted (Figure 6.6). The MDS of lipid peroxidation suggested that only Bloubergstrand is unpolluted (Figure 6.7). The criteria applied by Box *et al.* (2007), to classify sites as polluted and unpolluted using antioxidants as biomarkers is controversial and should be considered with caution.

The results of the present study are similar to others that measured antioxidant activities/levels in whole soft tissue of mussels who found that antioxidant activity/levels were significantly higher in polluted sites than non-polluted sites (Hultberg *et al.,* 2001; Lam & Gray, 2001; De Luca-Abbott *et al.,* 2005; Kakkar &

Jaffery, 2005; Gorinstein *et al*., 2006b). It is therefore proposed that antioxidant responses of *M. galloprovincialis* in the Cape Peninsula be considered as biomarkers of oxidative stress.

Considering the results of metals in the environment (Chapter 3) and previous records of metals in mussels at the same sites (Chapter 2 and Chapter 4), as well as the antioxidant responses in this chapter, it is suggested that Milnerton be considered a highly polluted site, Green Point moderately polluted site, Bloubergstrand and Hout Bay mildly polluted and Scarborough, an “unpolluted” site.

The Milnerton site is at the mouth of a stormwater pipe, situated at shore end of a range of hotels. Within the vicinity is an industrial area, including petro-chemical industries that could have been contributors of pollutants. It was also evident at the time of sampling that the water from the pipe was warmer than the surrounding sea water and this too may have been a contributing factor to the higher antioxidant activities/levels recorded. According to Jena *et al*. (2009), temperature also plays a role in affecting antioxidant activity in mussels. Bloubergstrand is at the shoreward end of a fast-growing residential area and this could account for increasing pollution activities. The Green Point site is at a shoreward end of a filling station and close to a stormwater pipe that is source of runoff from residential and commercial activities. It is also within 1 km of the Cape Town soccer stadium. The presence of the various types of human activities could result in an array of anthropogenic inputs at this site. Hout Bay is a historical fishing community with high tourism activity as well as shipping activity (both traffic and vessel maintenance). The site is at the shoreward end of commercial and residential activity, in particular at the seaward end of a squatter camp (an informal residential area). The site is also at the far end of an enclosed bay and the bay profile could result in current re-circulation of water which could result in retention of pollutants in the Bay. Scarborough is removed from residential, commercial and industrial activity and hence considered non-polluted.

To identify cause-effect relationships, the data from the metal and biomarker analysis, as described in Chapter four (section 4.3) and section 6.3 of this chapter were subjected to correlation analyses. Correlations between the metal concentrations and biomarker responses were calculated using the Pearson’s two-tailed correlation analysis and were applied to both biomarkers and metals

irrespective of sites. All the biomarkers showed some type of significant correlations with metals, with Cu having no correlations with any biomarker and Mn only having a significant correlation with ORAC. CAT was significantly, positively correlated with Fe, Zn and Cd, while SOD was significantly positively correlated Fe and Zn and GSH had the least significant positive correlation of the enzyme activities, being only significantly positively correlated with Fe. FRAP was the only biomarker with significant negative correlations with more than one metal, (Fe, Zn and Cd). ORAC were significantly positively correlated with Fe, Zn and Cd, and showed significant negatively correlation with Mn. CD was only positively correlated with Fe, whilst TBARS showed significant positive correlations with Fe and Zn.

6.5. Conclusion

The present study provides a preliminary account of antioxidant responses in mussels collected from five sites with different hydrographic and water quality status. The results indicate that there are significant differences in antioxidant responses at the five sites sampled suggesting that all the antioxidant activities applied, except CDs, are suitable for consideration as biomarkers of oxidative stress. It is suggested, based on the results reported, that antioxidant responses in mussels be considered as biomarkers of pollution in South Africa. However, it is recommended that a suite of biomarkers be considered in future research to determine responses to metal induced stress (antioxidant, DNA damage, metallothioneins, lysosomal membrane integrity). The advantage of using a combination of biomarkers is that they integrate effects from various pollutants, and act as an early warning of impacts at the community and population levels (Vasseur & Cossu-Leguille, 2003). More laboratory studies are needed that use cocktails of metals and other pollutants to improve understanding of cause and effect. Such cocktails of metals are seen as important mixtures of toxicity that provide better reflections of environmental concentrations and combinations of metals. Sampling of a variety of potential contaminants should hence be done to correlate the potential causes of higher antioxidant responses at sites that are considered to be polluted. The present results provide an account of changes in antioxidative responses of mussels collected from five sites with similar hydrographic features but different water quality status. Studies like this will provide essential

information in understanding whether antioxidant parameters serve as biomarkers of pollution and whether these could be incorporated into biomonitoring studies.

Table 6-1. Summary of Pearson's correlation coefficients between trace metals in mussels sampled and biomarker analysis done from the same sites. Figures in bold are statistically significant at $p < 0.05$ level.

	Mn	Fe	Cu	Zn	Cd
CAT (mmole/µg protein)	-0.267	**0.721**	0.161	**0.640**	**0.573**
SOD (U/mg)	-0.066	**0.746**	0.113	**0.551**	0.289
GSH (µmol/g)	0.228	**0.657**	0.086	0.323	-0.200
FRAP (µmol/g)	-0.013	**-0.390**	-0.303	**-0.429**	**-0.384**
ORAC (µmol TE/g)	**-0.477**	**0.574**	0.012	**0.506**	**0.451**
CD (mmol/mg protein)	0.227	**0.600**	0.074	0.278	-0.198
TBARS (µmol/µg protein)	0.044	**0.814**	0.112	**0.568**	0.168

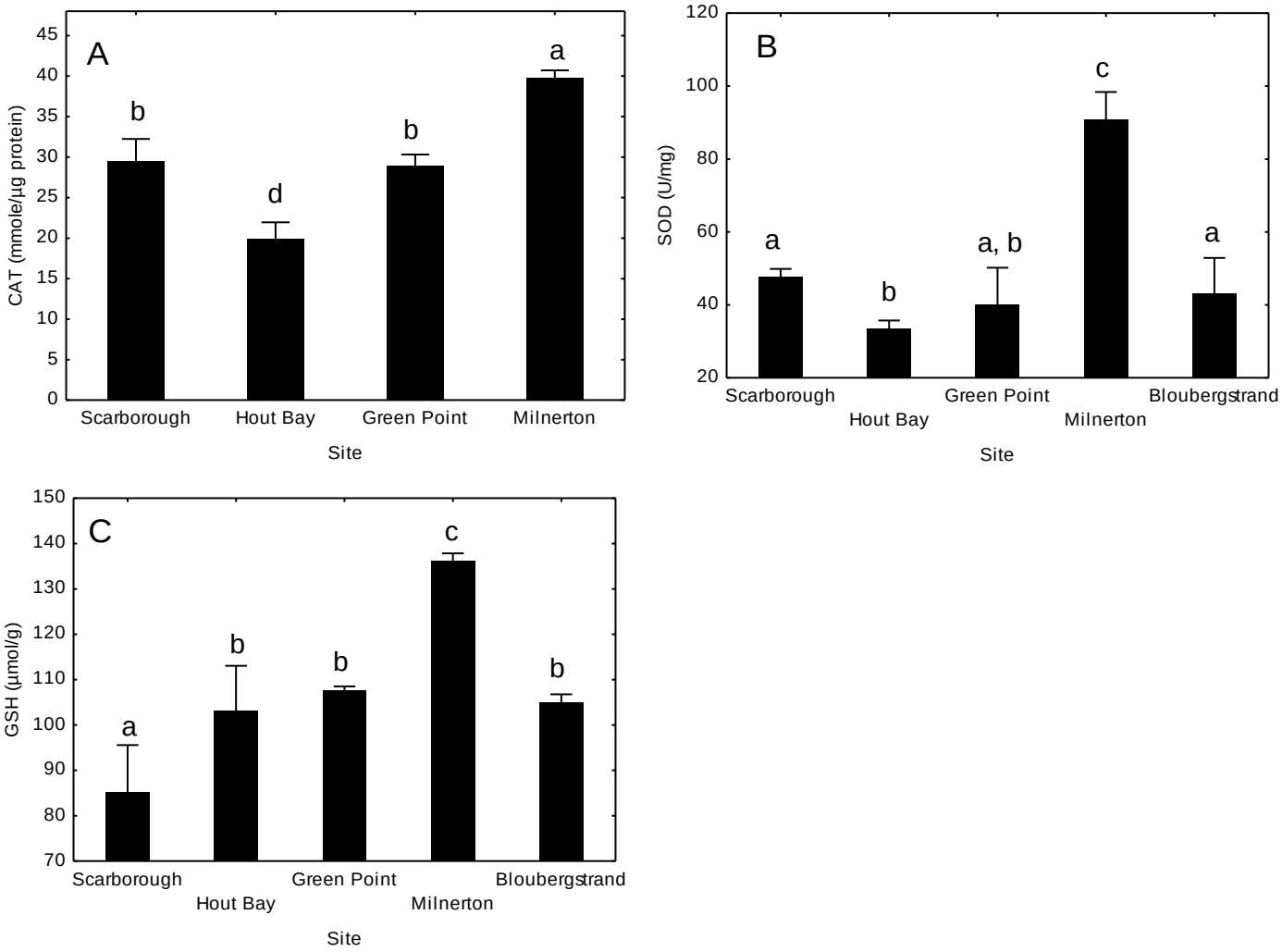

Figure 6-1. Mean antioxidant enzyme activities (± SE) in whole soft tissue of *M. galloprovincialis.* CAT (A), SOD (B), and GSH (C) activities were determined in all the studied sites. Statistical differences were determined using one-way ANOVA, followed by the Bonferroni post-hoc test ($p<0.05$). Different letters indicate significant differences between sites (n=5).

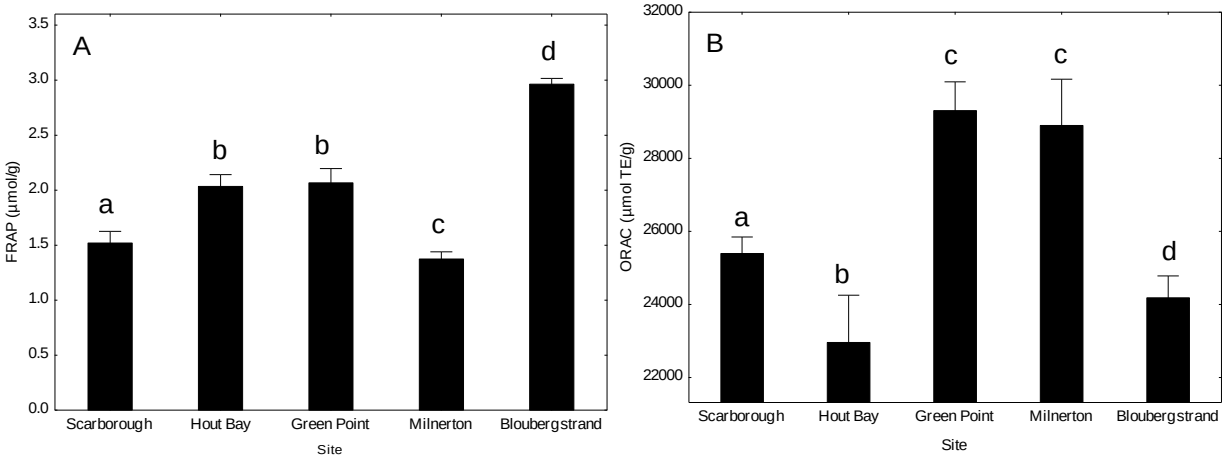

Figure 6-2. Mean antioxidant capacity (± SE) in whole soft tissue of *M. galloprovincialis.* FRAP (A) and ORAC (B) activities were determined in all the studied sites. Statistical differences were determined using one-way ANOVA, followed by the Bonferroni post-hoc test ($p<0.05$). Different letters indicate significant differences between sites (n=5).

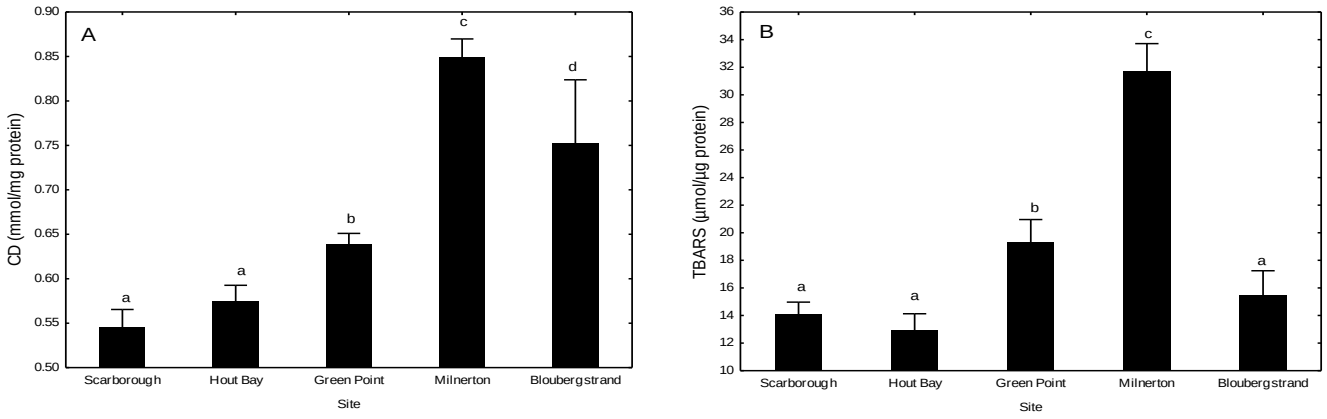

Figure 6-3. Mean lipid peroxidation (± SE) in whole soft tissue of *M. galloprovincialis.* CD (A) and TBARS (B) activities were determined in all the studied sites. Statistical differences were determined using one-way ANOVA, followed by the Bonferroni post-hoc test ($p<0.05$). Different letters indicate significant differences between sites (n=5).

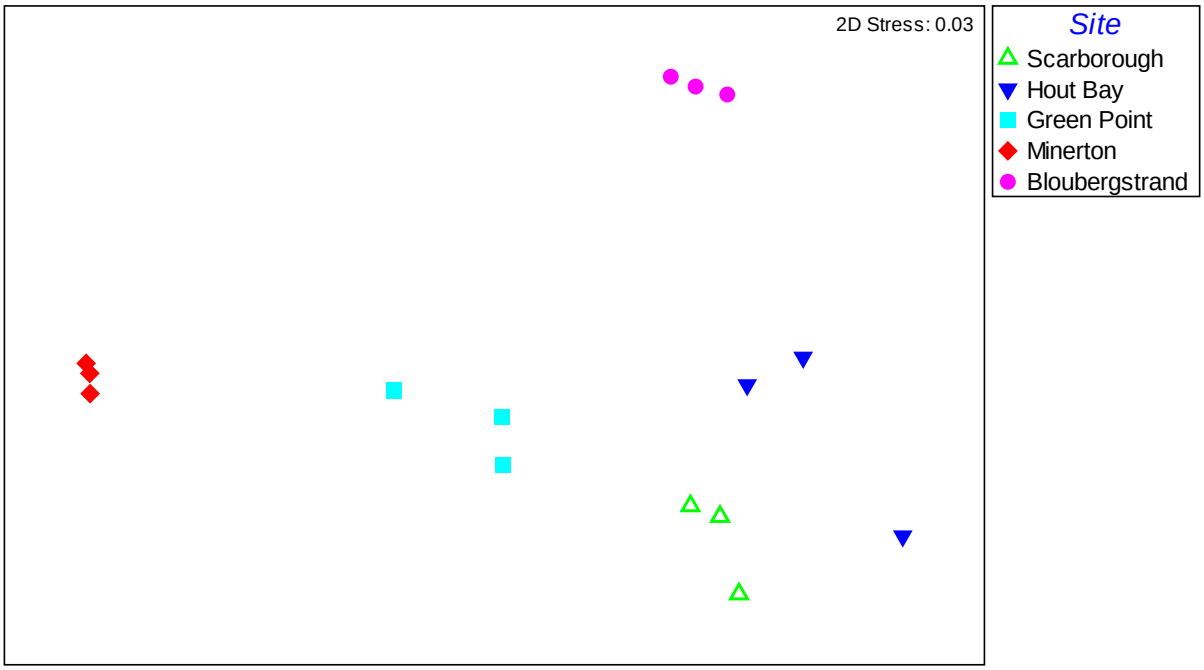

Figure 6-4. MDS of antioxidant response (all antioxidant activities) in *M. galloprovincialis* as determined at the various sites along the west coast of the Cape Peninsula, Cape Town.

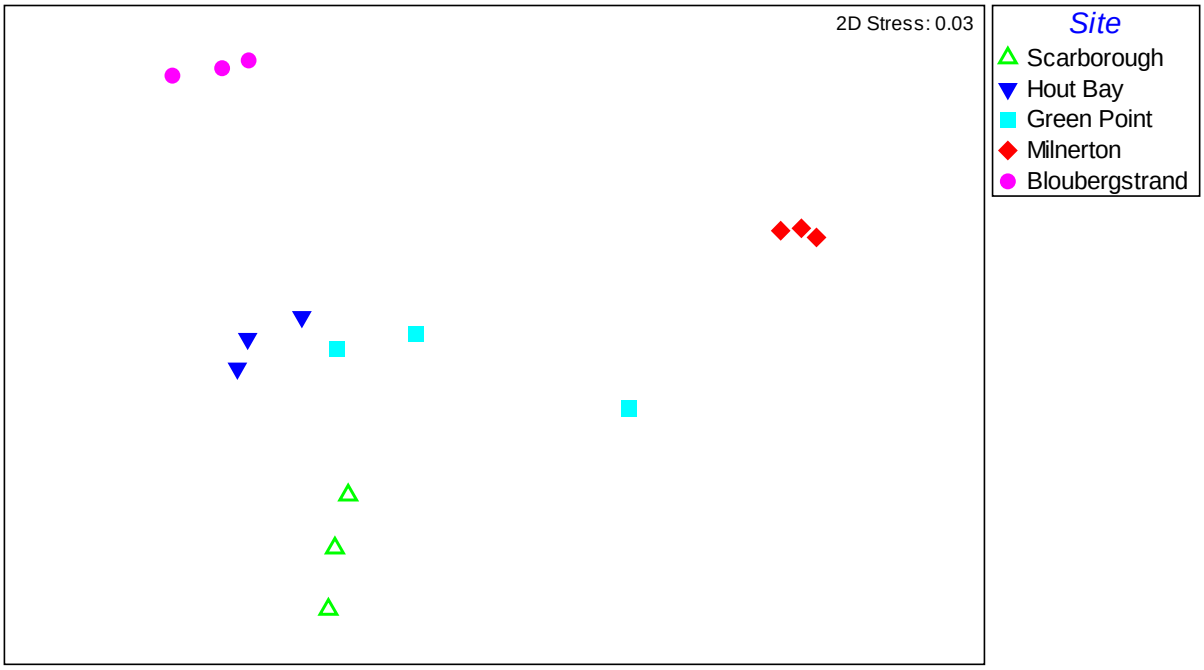

Figure 6-5. MDS of antioxidant level and enzyme activities (CAT, SOD and GSH) in *M. galloprovincialis* as determined at the various sites along the west coast of the Cape Peninsula, Cape Town.

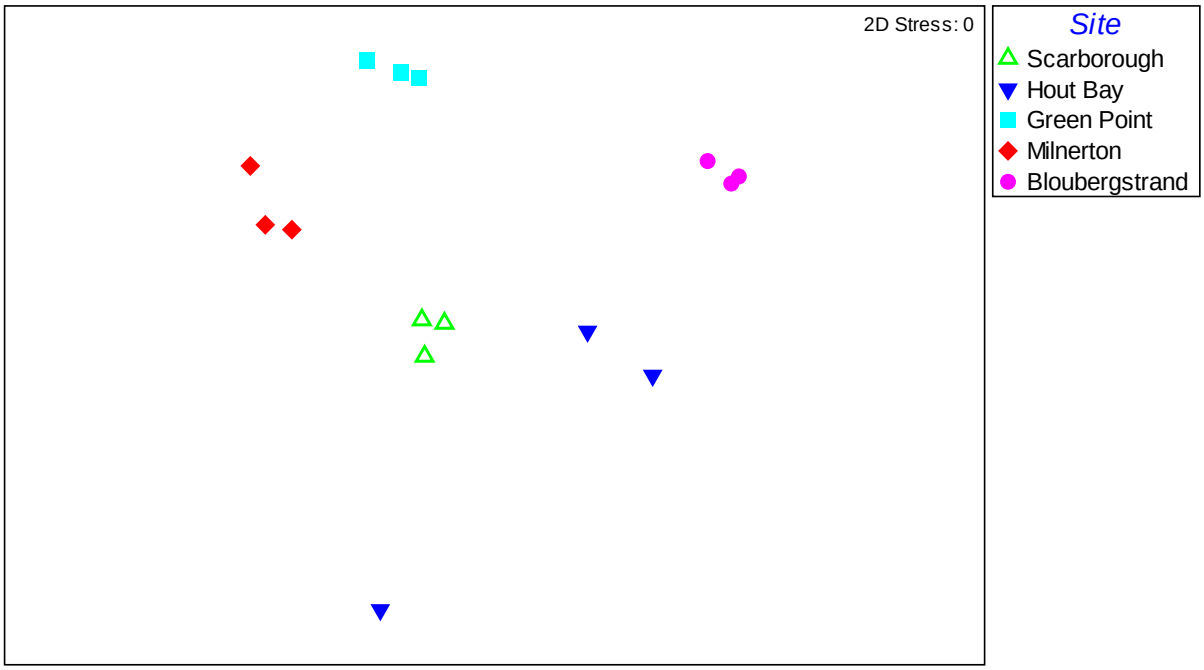

Figure 6-6. MDS of antioxidant capacity (ORAC and FRAP) in *M. galloprovincialis* as determined at the various sites along the west coast of the Cape Peninsula, Cape Town.

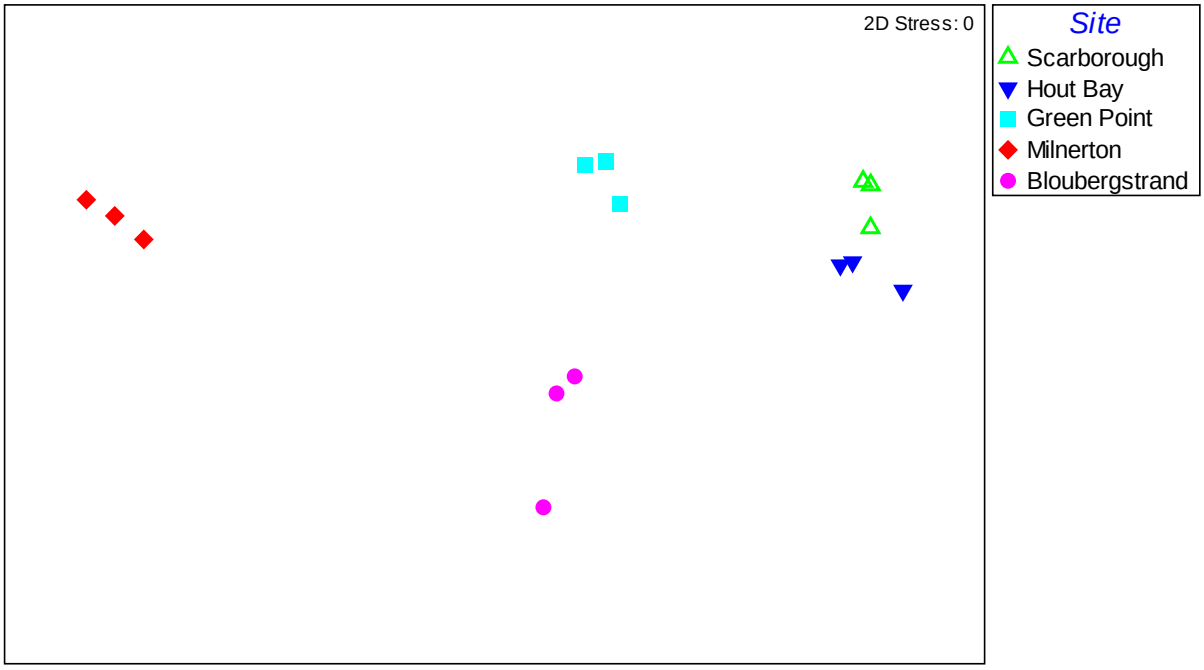

Figure 6-7. MDS of lipid peroxidation (CD and TBARS) in *M. galloprovincialis* as determined at the various sites along the west coast of the Cape Peninsula, Cape Town.

Chapter 7

7. General Conclusions

The research objectives of this thesis were to determine the level of metal accumulation along the west coast of the Cape Peninsula in the physical environment as well as in whole soft tissue of *Mytilus galloprovincialis*. Further, to ascertain to what degree metals were accumulating in the mussels and antioxidant responses could be used as biomarkers to determine the influence or impact of the metals.

7.1. Literature Review and Introduction

The introductory chapter considered the need for the present study by providing a literature review of marine pollution in South Africa and the Western Cape. This provided a basis to underpin the rationale and objectives of the study. The intention of the chapter was to provide an analysis of current knowledge pertaining to status of the marine ecosystem, marine pollution, ecotoxicology, biomonitoring and biomarkers as tools to ascertain the integrity of ecosystem functioning in the region. The review indicated that, although investigations into the levels of pollutants in marine environment are being conducted throughout the world, in particular the northern hemisphere, such investigations are lacking in southern Africa. Although considered to be in a pristine state, knowledge about levels and types of pollutants in the region is unknown. Also, Cape Town has one of the busiest ports in South Africa in Table Bay and the effects of maritime traffic on the coastal system have not been investigated. Furthermore, whether the growth in the metropolitan area has had an effect on the coastal system is not known. This introductory chapter outlined the need for knowledge about the health of ecosystems and discussed methods to ascertain the condition of ecosystems. Biomonitoring is being conducted throughout the world via a Mussel Watch Programme. Although the monitoring provides information about the levels of pollutants in mussels, it does little to determine the health status of organisms. The use of biomarkers could possibly address this shortcoming. In South

Africa, there are no accepted or established methods of using biomarkers in risk assessment. Hence, the objectives of the thesis were to determine the historical levels of metals by reporting on Mussel Watch Programme data of the study area; conducting sampling of the metals in seawater, sediment and mussels in the area; exposure of mussels to various doses of a metal to ascertain if bioaccumulation occurs and if oxidative responses can be used as biomarkers to determine the effect of that metal on *M. galloprovincialis,* and to what levels, and; to ascertain if the biomarkers used in the laboratory can be applied (validated) to field conditions.

7.2. A review of Mussel Watch Programme data from the west coast of the Cape Peninsula, Cape Town

Chapter 2 described the contamination levels of metals in *Mytilus galloprovincialis* sampled as part of the Mussel Watch Programme (MWP) data. The aim of the study was to report on the temporal and spatial changes in metal concentration in the mussels collected as part of the MWP. *M. galloprovincialis* samples have been collected since 1985 from the entire South African coastline (*Perna perna* is sampled along the east coast) and analysed for metal bioaccumulation. The results of the study showed that metal bioaccumulation in *M. galloprovincialis* were highly variable along the five sites sampled. The concentration of metals at all sites had highly significant differences between all metals for the period 1985 to 2008 ($p<0.001$). The mean order of metal concentrations from all sites combined were Zn>Fe>Cd>Cu>Mn>Hg. The general trend for Cu, Cd, Pb and Zn was a decrease in concentration from 1985 to 1991, an increase in 1992, and then metal concentrations gradually decreased towards 2005. Seasonally, Cd, Fe, Pb, Mn and Zn were higher in autumn 2010 whereas Hg was higher in spring 2010 and there was no evident seasonal trend in Cu. Although these were the general trends for all sites combined, it represented the general trend at the respective five sites analysed. A multivariate analysis revealed that there were significant effects of metal concentration on years and site as well as interaction between year and site. The results of the study suggested that metal bioaccumulation in *M. galloprovincialis* could have significantly influenced the organism negatively and further analysis of the nature of the effects of metals on *M. galloprovincialis* in the region is proposed.

7.3. Metal concentrations in intertidal sediment and waters off the west coast of the Cape Peninsula

In Chapter 3, the level of metals in the intertidal waters and sediment were measured at five sites along the west coast of the Cape Peninsula from August 2010 to August 2011. The aim of the investigation was to measure the levels of metals in the intertidal waters to consider the impact the metals may have on the area. The results of the investigation showed that sediment metal concentrations have increased only slightly over the past 30 years with only Zn and Cd at Bloubergstrand and Cu at Hout Bay increasing over the period (CF>50). The results showed that the CF was highest in winter 2010 (CF=20.79) and lowest in autumn 2011 (CF 2.58). The PLI index of metals in the sediment showed that the order of polluted sites were Hout Bay > Bloubergstrand > Green Point > Milnerton > Scarborough. The results presented hence supported the assumption that Scarborough was the least polluted site and provided support for it to be considered a control site for comparison purposes.

7.4. The bioaccumulation of metals in *Mytilus galloprovincialis* off the west coast of the Cape Peninsula

Chapter 4 investigated the bioaccumulation of metals in *M. galloprovincialis* from five sites along the west coast of the Cape Peninsula. The aim of the study was to collect data on metal concentration in the soft tissue of mussels and determine if any temporal or spatial trends were evident. The study found that most metals were significantly lower in mussels collected at Scarborough and that metals at most of the other sites were significantly different between seasons, with winter 2010 showing the highest significant difference to the other seasons probably due to water runoff from contaminated areas or from natural sources. There was a significant correlation between all metals measured irrespective of site and season. The efficiency of metal accumulation was measured using the BSAF. The results showed that the BSAF was highest for Cd, Pb, Zn and Cu with the lowest BSAF reported for Fe and Mn. This result suggested that *M. galloprovincialis* accumulated Cd, Pb, Zn and Cu at higher rates than the other metals, and could support the notion that these metals be ideally suited for biomonitoring. The results indicated that metals in the region were

bioavailable for uptake, but these were site specific. Despite the variability of the data reported, an overall pattern suggested that the order of magnitude of metal accumulation (or affinity for accumulations) was Fe>Cu>Cd>Zn>Pb.

7.5. Antioxidant responses in *Mytilus galloprovincialis* exposed to copper under laboratory conditions and their potential as biomarkers of metal exposure

The fundamental requirement for a monitoring organism is that it should be able to accumulate a pollutant in a predictable manner. To ascertain this, a predictable correlation should exist between the metal content of the organism and the concentrations in the environment it occurs in. The experiment described in the present investigation was intended to determine if/whether there was a relationship between Cu in accumulation in *M. galloprovincialis* and antioxidant responses to the levels of Cu exposure. The results of the experiment indicated that the copper accumulated in *M. galloprovincialis* over the 21 period exposure to 40 µg/L (low dosage) and 100 µg/L (high dosage) dosages. Mussels exposed to low dosages of Cu resulted in a 4 x increase in Cu concentration in its tissue, whereas mussels exposed to high dosages of Cu resulted in a 10 x increase in Cu concentration in its tissue.

The main aim of the present investigation was to test the potential of a series of antioxidant responses in mussels exposed to Cu to be used as biomarkers of metal-induced stress. To meet the aims of the study, total antioxidant capacity was measured using FRAP and ORAC, enzyme activity was determined using CAT, SOD and GSH and lipid peroxidation was determined using TBARS and CDs. The results showed that *M. galloprovincialis* exposed to high dosages of Cu had significantly increased antioxidant activities assumed as a response to the high dosages of Cu that the organisms were exposed to. However, the responses were not conclusive for all the antioxidant activities. For GSH, ORAC and TBARS, the antioxidant activities by day 21 were significantly different from that of the start of the experiment. There were significant differences between exposure groups and times, but these differences did not change over time. This suggested that *M. galloprovincialis* had different levels of antioxidant responses, some of which are considered to be as the

result of high Cu-induced stress loads. The end result was reduced antioxidant activity due to loss of the ability to function, rather than low impact of the stress. It was concluded that the responses could possibly be used as biomarkers, but field validation or application is necessary.

7.6. Antioxidant responses in M*ytilus galloprovincialis* as biomarkers of metal-induced stress along the west coast of the Cape Peninsula, South Africa

Chapter 6 investigated antioxidant responses in *M. galloprovincialis* collected from five sites along the western coast of the Cape Peninsula. The objective of the study was to assess the potential of antioxidant responses of mussels as biomarkers of oxidative stress under field conditions. To meet the objectives, biochemical activities and responses of the antioxidant system of mussels were used. Specifically total antioxidant capacity was determined using FRAP and ORAC, enzyme activity was determined using CAT, SOD and GSH and lipid peroxidation was determined using TBARS and CD's. The results showed variable antioxidant responses at the different sites, making interpretation of the biomarker activities difficult. The results did, however, suggest that based on antioxidant enzyme activity, Milnerton be considered a polluted site. The antioxidant capacity biomarkers suggested that Green Point, Milnerton and Bloubergstrand be considered polluted. The lipid peroxidation responses suggested that Milnerton and Bloubergstrand be considered polluted. Based on these finding it was recommended that Milnerton and Bloubergstrand could be considered polluted, Hout Bay and Green Point mildly polluted and Scarborough, unpolluted. Correlation analyses also showed that that all biomarkers had positive correlations with all metals, except Fe. There were no significant correlations between Cu and any of the biomarkers. It is recommended that further studies be conducted on biomarker responses as the results of the present study could not provide conclusive causes for the antioxidant responses reported. However, it is also noted that under field conditions, many factors contribute towards oxidative stress, including physical and biological factors. Compared to international standards, none of the sites sampled are considered polluted, but by using oxidative stress, a comparative level of stress, possibly, metal induced, was illustrated for the Cape

Peninsula. A suite of biomarkers are suggested for future investigations as well as measuring other sources of contaminants, such as organic pollutants.

The present study quantified the concentrations of metals in the water and sediment. It also considered the historical concentrations of metals in *M galloprovincialis* from 1985 to 2008 based on MWP data and also compared temporal and spatial metal concentrations in the water, sediment and mussel. These concentrations were then considered and recommendations made for the potential use of the mussels as biomonitors for biomonitoring, by considering the levels of bioaccumulation. It is suggested that a biomonitoring system be established in the Western Cape that uses a combination of monitoring tools (pollutants in the environment and organisms).

M. galloprovincialis was exposed to various doses of Cu and the potential of using antioxidant responses to metal accumulation as biomarkers was considered based on responses in a controlled setting. The biomarker responses were then validated by antioxidant responses in mussels sampled from five sites along the west coast of the Cape Peninsula. The responses of *M. galloprovincialis* to different Cu exposure levels requires further investigation, both in a controlled setting as well as mussels sampled from the wild. Although the mussels bioaccumulated metals, factors affecting the rate and site of accumulation within the organism requires further investigation. The knowledge gained by the present investigation does not warrant predictions to be made about the nature of bioaccumulation in *M. galloprovincialis*, but rather provides evidence for the need for more, detailed investigations, to be made about the nature of contaminations and the antioxidant responses to these contaminants in marine organisms. Future investigations should consider mixtures of toxicity by using exposure to variable types and quantities of pollutants to reflect the condition of the natural environment. The research in this thesis was however novel, in that it was the first study on antioxidant responses in mussels in the region and the results suggest that there is potential to use antioxidant responses as tools for monitoring the health status of the coastline of southern Africa.

Chapter 8

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