Acute and chronic toxicity of Copper and Zinc and environmental tolerances to the estuarine amphipod, *Melita zeylanica*.

by

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DECLARATION

I hereby declare that this dissertation is the product of my own original work, and to the best of my knowledge, contains no material previously submitted for the award of any degree at another university. Any published information was duly acknowledged.

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By Ms. R.P. Mofokeng

Dedication

I dedicate this Dissertation to:

My God without whom the completion of this thesis would not have been possible.

My family, without your love and support, completion of this thesis would not be possible. To my mom, thank you for the countless hours on the phone, words of encouragement and constant prayer.

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Abstract

Owing to economic growth and their geographic positioning, harbours and estuarine ecosystems have seen considerably accelerated development around them over the past years. This has in turn induced excessive stress on these systems. Due to elevated metal deposition from surrounding industries, toxic metal concentrations are often evident in these systems as sediments tend to accumulate metals. Toxicity tests are essential in predicting the effects of contaminated water and sediments on biota. Acute bioassays refer to short term bioassays with mortality as an end-point, while chronic bioassays often take longer with sub-lethal end-points including growth and fecundity. In the past, acute toxicity tests were often reported as they are easier to conduct and generally more affordable than chronic tests. Chronic bioassays, however, are more relevant as chronic exposures are most likely to occur in the environment and they have higher sensitivity to metals. Sediment toxicity tests are often preferred over water-only tests, as metals tend to bind to sediment over prolonged periods of time, therefore, they provide a time-integrated measure of metal accumulation within estuarine ecosystems.

Due to their high metal toxicity, environmental tolerance, abundance, as well as ease to handle in the laboratory, amphipod bioassays are used worldwide in toxicity testing. In this study acute and chronic toxicity bioassays were undertaken with the aim to determine the suitability of *Melita zeylanica* as an estuarine benchmark toxicity test organism in sub-tropical ecosystems. *Melita zeylanica* amphipods were collected in Mzingazi Canal, Richards Bay Harbour, and cultured in climate controlled chambers in aerated culture trays. Exposure experiments of *M. zeylanica* to Copper (Cu) and Zinc (Zn) were conducted at 25^0C and a salinity of 25 using a 6 by 5 grid. The LC_{50}, EC_{50} as well as metal concentrations in tissue and sediment were determined following a microwave digestion method, while an ICP-MS was used for metal analysis of sediment and tissue concentrations.

An overview on amphipod toxicity bioassays using Cu and Zn over the past 10 years (2003-2013) was conducted with the aim to report on chronic toxicity publications during the past decade. General sensitivity of amphipods to Cu and Zn as well as the frequency of use of various endpoints (fecundity, accumulation, growth and behaviour) were reported. Results of the overview suggested that amphipods are generally more sensitive to Cu than Zn. *Corophium volutator*, however, showed a lower LC_{50} value for Zn (10.03 µg.g-1) than that of Cu (26.07 µg.g-1). Fecundity was the most reported endpoint with *C. volutator* being the most used

amphipod over the past ten years. It was concluded, however, that no one amphipod species can represent all amphipod species as amphipods tend to adapt deferentially to their immediate environment.

A Cadmium (Cd) reference toxicity test is often used as a method of standardising inter-laboratory results as it is non-essential in organisms. A Cd 96hr static water-only reference test was used in this study to determine relevant toxicity of *M. zeylanica* as compared to other amphipods. This study also evaluated the median lethal concentrations (LC_{50}) of ammonia on *M. zeylanica* during a static 96hr sediment toxicity bioassay. Survival of *M. zeylanica* across a broad range of salinity (5-40) was also determined during a 96hr bioassay. The Cd water-only tolerance test showed that *M. zeylanica* sensitivity to metals is comparable to that of other amphipods, with a LC_{50} of 1.17 µg/g. *Melita zeylanica* was found to be more sensitive to ammonia as compared to other studies with a LC_{50} of 17 µg/g. *Melita zeylanica*, however, was found to be tolerant to a wide range of salinities (5-40) but intolerant to freshwater.

A 10 day acute sediment toxicity test with mortality as an end-point and a 28 day chronic sediment toxicity test, with growth and fecundity as endpoints, were conducted. Following the acute toxicity test, LC_{50} values for Zn and Cu were found to be 9.15 and 11.76 µg/g, with LC_{90} values of 238.5 and 78.6 µg/g showing that *M. zeylanica* is more sensitive to Cu at high concentrations .Tissue metal levels showed that *M. zeylanica* is able to accumulate both Zn and Cu in relation to sediment concentrations. This finding demonstrated that *M. zeylanica* is potentially a good biomonitor organism, as the ability to accumulate metals is one of the key requirements that qualify organisms as toxicity organisms. The chronic bioassay showed that both Zn and Cu affected fecundity of *M. zeylanica*. Amphipods were, however, found to be more sensitive to Cu compared to Zn. EC_{50} values of 2.7 and 0.8 µg/g were recorded for Zn and Cu, respectively. Amphipod growth was also inversely correlated to Cu concentration.

South Africa has experienced accelerated growth in its economy over the past decade which has resulted in increased demand for cargo container handling capacity throughout South African ports. Richards Bay Harbour is currently the largest deep-water port in South Africa, used primarily for the export of coal. Furthermore, there have been proposals to expand the port over the next 40 years, resulting in a 5 times increase in its surface area. In order to test the suitability of *M. zeylanica as* a bio-indicator of estuarine contaminated sediments in South Africa, a field validation study was conducted in Richards Bay Harbour with sediment samples being collected from 9 sites. Samples were analysed for aluminium (Al), Arsenic (As), Cd, Cu,

Iron (Fe), Mercury (Hg), Nickle (Ni), Lead (Pb) and (Zn) concentrations. Metal concentrations within the harbour showed significant spatial variation with the highest concentrations recorded at the Bulk terminal. Metal concentrations were found to be within standard quality guidelines as stipulated by the Department of Water Affairs as well as the Australian sediment guidelines. The data from this study was compared with historical data to identify contaminated areas. Elevated Zn concentrations within the harbour were contrary to historical data, as a result Polycyclic chlorine biphenyls (PCB) and Polycyclic Aromatic Hydrocarbons (PAH) analysis was conducted to identify or eliminate oil-spill as a potential reason for the high Zn concentration.

Metal accumulation in *M. zeylanica* following exposure to sediments showed that *M. zeylanica* is a good accumulator of metal, particularly for As, Cr and Hg. When compared to other amphipods widely used in toxicity bioassays, *M. zeylanica* was found to accumulate metals to comparable concentrations. A range of 5-59 μg/g and 98-227 μg/g was recorded for Cu and Zn, respectively which was comparable to that of *Gammarus oceanicus* (14-28 μg/g Cu) and higher than that of *Melita matilda* (140 μg/g Zn), a closely associated species.

Richards Bay Harbour is of high ecological and economic importance as it serves as both a fully functional estuarine ecosystem and is also one of the busiest ports in South Africa. Richards Bay Harbour as an estuarine environment has already been put under immense pressure with increased cargo handling over the past years. Plans to expand the harbour will also result in re-suspension of metals causing secondary metal contamination, which may contribute to loss of organisms and thus hinder the role of the harbour as an ecosystem. Although several toxicity and biomonitoring studies have been conducted on the harbour, no Estuarine Management Plan (EMP) has been put in place for Richards Bay Harbour as yet. An EMP should, therefore, be urgently designed and implemented, with all stakeholders accommodated.

National Sediment Quality Guidelines (SQG's) should also be implemented as different organisms in different areas do not necessarily react in the same manner, therefore, the use of international SQG's would not be entirely appropriate. In terms of Estuarine Ecological Biodiversity, Richards Bay Harbour is classified as a category C estuary, according to the National Water Act; no estuarine system should be allowed to degrade below D-class. The intended development of Richards Bay Harbour will, potentially, result in deterioration of the ecological integrity of the harbour. An EMP is thus of high importance within the harbour given the divergent roles of Richards Bay Harbour as both an estuarine ecosystem and a large cargo handling port.

Although *M. zeylanica* was found to be sensitive to ammonia, it was overall, found to be a good accumulator of metal, relatively easy to handle in the laboratory, tolerant to a wide salinity range and it was found in abundance in the Mzingazi Canal. Furthermore, accumulation of metals within amphipods did reflect sediment metal concentrations, particularly for Zn and Cu, following the 10 day toxicity bioassays. The findings of this study, thus suggest that *M. zeylanica* can potentially be used as a benchmark toxicity test species in sub-tropical estuaries in South Africa and can be recommended as a suitable biomonitor species in South African coastal waters.

Key words: **Amphipod, Acute, Chronic, Estuary, Overview, Richards Bay Harbour, Sediment, Toxicity.**

Table of contents

List of Tables

List of Figures

List of Abbreviations:

Bulk (C4): Bulk Terminal Site 4

Bulk (C7): Bulk Terminal Site 7

Dw: dry weight

ERL: Effects Range Low

ERM: Effects Range Medium

GC: Gas Chromatograph

ICP-MS: Inductive coupled Plasma-Mass Spectrometer

IUPAC: International Union of Pure and Applied Chemistry

LOD: Limit of Detection

N/F: Not Found

NIST: National Institute of Standards

MDL: Method Detection Limit

PAHs: Polycyclic Aromatic Hydrocarbons

PCBs: Polycyclic biphenyls

RBCT (5): Richards Bay Coal Terminal Site 5

RBCT (6): Richards Bay Coal Terminal Site 6

SQG's: Sediment Quality Guidelines

SD: Standard Deviation

USEPA: United States Environmental Protection Agency

CHAPTER 1

INTRODUCTION

1.1 Introduction

Going green and global warming have become regularly used terms. These terms emphasise the important fact that man has caused substantial harm to the environment and, therefore, measures need to be taken to conserve and protect our natural ecosystems. Activities such as mining, industrialization and urbanization have resulted in considerable change in the composition of the earth's chemistry as we know it (Elliot and Quintino 2007), with chemical pollution being one of the major challenges facing the global ecosystem (An and Kambel 2003).

Heavy metals can enter the aquatic system in numerous ways and these include; the atmosphere, point and non-point sources from industrial areas and land runoff. On entering aquatic ecosystems, metals either remain suspended in the water or they settle and eventually accumulate on the sediment where they become bioavailable to epibenthic organisms. These heavy metals, therefore, eventually impact on biodiversity as well as on the integrity of aquatic ecosystems (Chapman 1995). Heavy metals are potentially detrimental for aquatic organisms as they tend to bind to any molecule with an affinity for sulphur and nitrogen (Wikkie *et al.* 2010). Because proteins are made up of amino acids which contain both sulphur and nitrogen, they tend to concentrate large quantities of trace metals (Rainbow 2002). It is thus expected that there will be an abundance of binding sites for trace metals since cells are predominantly made of proteins. This, therefore, implies that organisms have a potential to accumulate metals to highly toxic levels in their bodies.

1.2 Trace metals

The terms "trace metal" and "heavy metal" are often used interchangeably; essentially, trace metals are heavy metals that are required by organisms in small (trace) amounts. Some trace metals such as Cd and Pd, however, are not essential to the body yet they are important in aquatic systems due to contamination. Metal classification according to affinity was described by Nieboer and Richardson (1980), who stated that metals could either belong to Class A (metals which bind with ligands with oxygen as a donor atom), Class B (metals which bind with ligands with sulphur as a donor atom) or Class C (metals can show intermediate behaviour between class A and class B). Most trace metals are classified as Class B (Nieboer and Richardson 1980).

According to Rainbow (2007), living organisms often accumulate trace metals in their tissues regardless of whether these trace metals are required by the organism. Rainbow (1995)

reported that marine organisms have a tendency to store trace metals to high concentrations, which accumulate in their adipose tissue and therefore, the metals do not affect the organism until the organism requires extra energy from its adipose tissue. These accumulated metals are easy to measure and provide a more or less accurate concentration of the metal as well as time integrated measure of metal supply over time (Rainbow 2006). .

Since benthic organisms such as amphipods can deposit feed as well as suspension feed, they are exposed to metals associated with feeding on overlying particles, pore water as well as sediment particles (Simpson and Batley 2007).

1.3 Estuarine contamination

Defining an estuary is not necessarily a simple concept as there are a number of factors to take into consideration (Whitfield 1989), these include, the opening and closing of the mouth, tidal influence, salinity as well as geographical characteristics (Vivier 2010). Although there are many proposed definitions of estuaries, the one most accepted was proposed by Day (1980), and states that an estuary is "a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of sea water with freshwater derived from land drainage".

Estuaries are the immediate receptors of contaminants from runoff and rivers and they also form a link between fresh and salt water. Estuaries, therefore, have strong gradients in many physical and chemical variables i.e. salinity, temperature, pH, dissolved O_2, redox potential and nutrients (Chapman and Wang 2001).

Estuarine ecosystems are among the most productive ecosystems in the world (Vascocelos *et al.* 2007). The nutrient rich environment of estuaries accounts for its rich biodiversity, which makes estuaries an ideal environment for fisheries (Begg 1978). Estuaries function as important nursery areas for many marine fish and invertebrate species due to the relatively sheltered waters and high nutrient load, which include many economically important species (Cyrus 1991; Cyrus 2001; Vivier 2010). Vivier and Cyrus (2001) also emphasised the importance of estuaries worldwide as nursery environments for juveniles of estuarine-dependent marine fish.

Estuaries are regarded as important ecological systems and they depend largely on rivers for freshwater input (Cyrus 1991). According to Whitfield (1989), the natural functioning and diversity of estuaries can only be maintained through regular contact between the estuarine and marine environment.

Increasing anthropogenic influence has a considerable effect on estuarine and inshore ecosystems worldwide. There is substantial evidence that most estuarine ecosystems are under stress, mainly due to increasing industrial and residential development around their fringes (Elliot and Quintino 2007). This then results in elevated toxicity levels within aquatic systems.

1.4 Toxicity testing

1.4.1 Importance of development of toxicity tests

Toxicity tests are important in predicting the effects of contaminated water and sediments on biota (Gale *et al.* 2006), and for this reason, toxicity tests, particularly sediment toxicity tests, have proven appropriate in ecological monitoring studies as they provide knowledge and understanding of the sensitivity of organisms to contaminants and also incorporate a time factor (Rainbow 2007). Ecotoxicology aims to primarily identify and solve topical problems concerning environmental health, but more essentially, it aims to prevent toxicity related issues and therefore should be part of a broader decision making framework connected to national policy.

Some of the more important reasons that toxicity tests be developed are;

- Contaminants present in an ecosystem remain a potential risk to organisms that rely on sediment for food, as habitat and as a refuge and will ultimately affect ecosystem and human health (Wang and Rainbow 2008).

- Water quality guidelines have been extensively applied over the past years in ecosystem monitoring and not as much attention has been given to sediment toxicity tests, particularly in South Africa. Sediment toxicity tests are frequently used to assess the potential impact of contaminated sediments (Kennedy *et al.* 2009) and it is becoming more and more important to be able to use accurate sediment quality guidelines (SQGS) to determine relative levels of contamination (Simon and Batley 2007).

1.4.2 Toxicity testing in Estuaries

Estuarine ecotoxicology evaluates toxicity levels in estuaries and their impact on estuarine biota. Ecotoxicology is used essentially to differentiate pollution from contamination, where contamination refers to chemical increase, while pollution refers to substantial harm to organisms (Chapman 1995). Although, generic guidelines are often the most used methods for ensuring some level of protection for organisms in the environment, more studies focusing on ecologically relevant tests with native organisms such as amphipods and the field validation of

guidelines are still required (Melo and Nipper 2005).

Most sediment toxicity studies, however, are dedicated to freshwater and marine water with much less reference to estuarine waters (R´e *et al*. 2009). Using estuarine organisms will be much more informative and accurate for estuarine environments than the results obtained using marine organisms as these will more accurately reflect activities within estuaries (Lawrance and Poulter 1997). There, however, is an increasing shift in recent years as more recent data tend to report on estuarine species (Edwards *et al.* 2003; R´e *et al.* 2009; DeLorenzo *et al.* 2014). In South Africa, however, estuarine toxicity testing is still in its infancy and relevant information is limited.

1.4.3 Sediment toxicity testing

Sediment toxicity tests are an important tool for predicting the effects of contaminated sediments on biota (Gale *et al.* 2006). Sediments are the final destination for contaminants entering aquatic systems and concentrations of metals in sediments are often several magnitudes higher than aqueous concentrations (An and Kampbell 2003). Sediments are the main sink of trace metals and also form an important storage and transport medium for potentially toxic metals (Alonso-Castillo *et al*. 2013; Superville *et al*. 2014). According to Zhao *et al*. (2010), different aquatic systems are affected by different land uses. Metal elements from various sources are deposited onto sediment surfaces and are eventually incorporated into the structure of minerals within the sediment (e.g. Fe-Mn oxides). Huo *et al*. (2013) also stated that only minute concentrations of free metal ions remain dissolved in water, while about 90% adheres to sediments. Given the ecological and economic importance of estuarine environments (Day 1980; Chapman 1998; Rainbow 2006), it is expected that a substantial number of studies have been conducted on metal contaminants within these systems. Knowledge of the partitioning and distribution of metals in sediments provide insight into both the source of contaminants as well as their effects on aquatic biota. Historically, toxicity evaluations were largely based on chemical analyses together with a comparison of unpolluted environments (Casado-Martinez *et al.* 2007). Sediment toxicity tests have provided a methodology for obtaining a more meaningful understanding of the dynamics of heavy metals particularly in relation to their origin, duration within the aquatic system as well as partitioning (Alonso-Castillo *et al.* (2013).

1.5 Acute vs. chronic toxicity test

Toxicity tests include acute tests with mortality endpoints or chronic toxicity tests with sub-lethal endpoints (Kennedy *et al.* 2009). In terms of test duration with regards to the life-cycle of the test species, acute bioassays are those that span a relatively short period whereas chronic bioassays are those where the exposure lasts most of the life-cycle and permits evaluation of sub-lethal effects (Ceaser *et al.* 2004). Acute toxicity is essentially a method used to determine the concentration of a chemical that has a deleterious effect on organisms in a short period of time (e.g. 96 hours), with mortality mostly being the endpoint (Walum 1998). Castro *et al*. (2006) reported that because they are relatively easy to conduct and have a short experimental span, acute tests have historically been the most conducted. Costa *et al*. (2005) also emphasised that, fewer chronic tests have been implemented compared to acute tests as the former procedures are often lengthy and tedious, which makes them less desirable in most monitoring programs. There has been progress in the last 10 years with chronic studies being increasingly reported. In the USA and Australia, for instance, *Leptocheirus pulmulosus* and *Melita plumulosus* have been studied quite extensively, to name but two examples (King *et al.* 2006a; Manyin and Rowe 2006; Hanna *et al*. 2013; Hook *et al.* 2014). The importance of chronic toxicity studies is being realized more and more and the following four statements form the basis of why this is the case (Costa *et al.* 2005):

a) Chronic exposure is much more likely and occurs more frequently than acute exposure, more so in natural environments.
b) Most sediments are moderately contaminated.
c) Biological effects of contamination other than survival may be more appropriate and meaningful when compared to acute effects as chronic effects are more relevant for interpreting community structure related impacts of toxins on organisms.
d) Chronic tests tend to have higher sensitivity and therefore are ecologically more relevant.

Although chronic bioassays may be more expensive and tedious than acute tests, they have more relevance at population level as opposed to acute tests which tend to focus more on the individual (Van den Heuvel-Geve *et al.* 2007). Scarlett *et al.* (2007) stated that although acute tests are valuable in identifying chemical toxicity, they do not take the entire life cycle of

organisms into consideration. The most important reason for using chronic toxicity tests, however, is that they are sensitive to sub-lethal effects i.e. at concentrations below which mortality occur, while acute toxicity tests focus on mortality as the end-point. Endpoints mostly used in chronic tests include growth and fecundity (USEPA 2001, van den Heuvel-Greve *et al*. 2007). Growth experiments are essential in that they provide insight into the life-cycle of organisms, i.e. if growth is delayed, the life-cycle takes longer, thus impeding maturation as well as the overall population dynamics of organisms (Van den Heuvel-Geve *et al*. 2007). In this study, growth and fecundity were evaluated as chronic toxicity endpoints in the estuarine amphipod, *Melita zeylanica*.

A literature review of the use of chronic toxicity tests in recent years was conducted as part of this study to investigate whether there has been an increase in the number of chronic toxicity testing studies (see chapter 2).

1.6 Why Cu and Zn?

Although some trace metals are required by the body in small quantities, when present in high concentrations, they can have a detrimental effect on animals and plants (Wikkie *et al.* 2010). Zinc is an essential trace element, however, at high concentrations it can have severe impacts on the survival of organisms (Rainbow 1995). Copper is also an essential micronutrient used in enzymes involved in several metabolic processes and is essential for growth and development. At high concentrations, however, Cu is one of the most toxic metals. It is also readily available in the environment, more so around areas of industrial development (Ytreberg *et al.* 2010). Since both Zn and Cu are essential elements, they are expected to vary in tissue concentration, because they are required in different quantities physiologically, especially between different genders (McPherson and Chapman 2000). Chakraboty *et al*. (2010) showed Cu to be more toxic than Zn but both Cu and Zn were highly toxic as they had strong interactions with biotic lignands. Copper and Zn were used in this study as both are regarded as contaminants of concern in industrialized estuaries such as Richards Bay Harbour (Wepener and Vermeulen 2005; Greenfield *et al*. 2011) and many studies have been done on the toxicity of these metals on estuarine crustaceans, notably amphipods (Chapman and Wang 2001; Marsden and Rainbow 2004).

1.7 Cadmium as a reference toxicity test

Cadmium is not an essential metal, therefore its accumulated concentrations are expected to vary only according to bioavailability differences (Conradi and Depledge 1998; Rainbow 1995) and its presence in organisms is proportional to that bioavailable in the environment. $CdCl_2$ is mostly used as a reference toxicity test as a way to define some standard of reference between bioassays. In this study the relative sensitivity of *M. zeylanica* to trace metals was determined using the 96hr static water-only Cd reference test.

1.8 Richards Bay Harbour

Harbour environments are typically exposed to numerous anthropogenic sources of contaminants as they are often surrounded by intensive industrial development. Cargo transport often leads to spillage and accumulation of contaminants within sediments (Weerts 2002). This is of concern as these materials may be deleterious to organisms naturally occurring in the harbour. Richards Bay was initially a shallow highly productive estuarine ecosystem surrounded by *Phragmites and Papyrus* swamps (Weerts *et al*. 2003). During port construction in the 1970's, a consensus was reached between maintaining the ecological integrity of the system and economic development through conversion of the northern section of the estuary into a deep water harbour, while the southern section (now Mhlathuze Estuary) was maintained as an estuarine bay ecosystem and became a conservation area, called the Mhlathuze Sanctuary (CSIR 2005). The two sections are divided by a 4km long berm. The lower reaches of the Mhlathuze River were diverted south to drain into the Mhlathuze Estuary.

Richards Bay Harbour is affected by industrial effluent discharges from a variety of heavy industries, such as aluminium smelters and fertilizer plants, which lead to contaminants entering the harbour. Furthermore, harbour activities such as shipping, storage, as well as loading and offloading of dry bulk cargo and coal also contribute contaminants into the harbour. It follows, therefore, that Richards Bay Harbour is susceptible to a variety of industrial contaminants, which may be detrimental to organisms inhabiting various habitat types in the harbour.

1.8.1 Ecological importance of the Richards Bay Harbour estuarine system

Despite being the largest and one of the busiest deep-water ports in South Africa (Greenfield *et al*. 2014), Richards Bay Harbour still contains large areas of natural habitat, including intertidal mudflats and mangroves, and has retained much of its ecological functioning. In the most comprehensive assessment to date of the ecological importance of South African estuaries,

Turpie *et al.* (2002) ranked Richards Bay 26th out of 250 estuaries in the country for conservation importance, 3rd for the ecological significance of its fish and bird communities, 5th for zonal type rarity, a score of 100% for estuarine size (based on relative size of estuarine area in the country) and a score of 85% for biological diversity. Regionally, when Richards Bay Harbour was compared to the 22 Zululand estuaries in KwaZulu-Natal north of Durban, the system ranked 6th for overall conservation importance, 2nd for zonal rarity, 8th for biodiversity and was among the four estuaries with a score of 100% for estuarine size (Turpie *et al.* 2002).

1.8.2 Development Priorities

Although recognised as an estuary of high conservation value and importance, future port expansion and industrial development is likely to place increasing pressure on the ecology of existing port areas (Greenfield *et al.* 2011). Increased dredging and activities associated with the development of the harbour will inevitably result in disturbance and destruction of ecologically important and sensitive areas coupled with the increased probability of contamination (Greenfield *et al.* 2011). Expansion of port cargo handling is set to continue as it has potential socio-economic benefits both locally and nationally. On the downside, the expansion also has potential ecological risks as it will unavoidably affect habitat distribution of organisms within and around the estuary. Dredging and sediment disturbance will also potentially affect a range of ecosystem components within the harbour.

1.9 Toxicity test organisms

Sediment toxicity tests have been shown worldwide to be an appropriate way of enhancing our understanding of organism sensitivity to contaminated sediments. Furthermore, sediment toxicity tests are valuable as they afford a time-integrated measure of the bioavailable fraction of contaminants, which may differ from the total amount present in the environment (Chapman and Wang 2001; Bat 2005). It is, therefore, important to take into consideration that different organisms respond differently to chemical exposure (Costa *et al.* 1998). While most organisms tend to accumulate metals in their adipose tissue, some organisms do regulate even minute metal contamination. For this reason, the choice of organism is also an important factor in toxicity testing (Conradi and Depledge 1998) as choosing the correct test organism directly influences the success and relevance of a test (Chapman and Wang 2001).

Only the bioavailable fraction is potentially toxic to organisms and is thus of ecotoxicological relevance in biomonitoring studies (Rainbow 1995). It is important, however, that organisms

which are closely associated with the sediments through burrowing and feeding habits, such as polychaetes and crustaceans, be used in toxicity testing. Although mussels are often used in toxicity studies, they do not incorporate the potential toxicity of contaminated sediments as they are filter feeders and are only ideal as biomonitoring organisms to investigate contamination of surface waters (Rainbow 2002). In the past years, standardized sediment toxicity tests using benthic organisms such as amphipods have become regular biomonitoring tools in New World and European coastal regions (Rainbow 2002; Marsden and Rainbow 2004).

1.9.1 Amphipods in toxicity testing

Amphipods are often used as biomonitors in aquatic environments as they are mostly sensitive to environmental disturbance as well as increased metal contaminants (Chapman 1998; Casado-Maritinez *et al.* 2007). Amphipods are said to be well suited for short term toxicity tests involving whole sediment and are strongly recommended as appropriate organisms for tests in the marine and estuarine environments (Cesar *et al.* 2004). They are sensitive to metals and are often highly abundant, thus easily accessible. Although some are tube-dwelling, amphipods are mostly epibenthic, or benthic feeders, therefore, they tend to be in contact with both sediment and pore water. They are also generally easy to handle in the laboratory and mostly reproduce throughout the year. Although amphipods generally reproduce all year long, they are known to increase reproduction output during the warmer months and are most abundant in temperate littoral regions (Krishnan and John 1974). Presently, bioassays using amphipods are regularly being used for toxicity management in estuarine ecosystems, particularly in developed countries (Casado-Martinez *et al.* 2007).

Amphipods have been reported to feed on a variety of food sources such as detritus and benthos, and they can also be scavengers as well as filter-feeders (Pennak 1978). They in turn are preyed upon by fish, invertebrates and birds and thus form an important part of the estuarine food chain (Krishnan and John 1974).

Arguably, the most important reason for amphipods being used in ecotoxicity studies is the fact that they are predominantly net accumulators of metals (Rainbow 2007) and therefore give a much more realistic interpretation of results than the chemical analysis of water.

1.9.2 Melita sp.

Amphipods of the genus *Melita* form an abundant part of the estuarine fauna throughout the world (King *et al*. 2006a; Hook *et al.* 2014; Hanna *et al*. 2013). There is not much data on melitid amphipods in South Africa. The most studied melitid amphipod to date is *Melita plumulosa* and there have been numerous reports regarding culturing and toxicity testing this amphipod (Hyne *et. al.* 2005). *Melita plumulosa* is highly tolerant to estuarine physico-chemical parameters yet highly sensitive to chemical contamination and is routinely cultured in laboratories and used in whole sediment toxicity tests (Hyne *et al.* 2005; Hook *et al*. 2014).

1.9.3 Melita zeylanica

There is limited information on *Melita zeylanica* in the literature. It is a gammarid estuarine species with an Indo-Pacific distribution, extending from India (Krishnan and John 1974), through Mauritius and Mozambique (Appadoo and Myers 2005) to southern Africa (Velasquez *et al.* 1991), including various estuaries along the east coast of South Africa (Barnard 1995).

Melita zeylanica is a euryhaline species found mainly in estuarine environments, but it does extend to freshwater as well and it breeds all year round (Appadoo and Myers 2005; Arovind *et al.* 1991). One of the early studies on *M. zeylanica* (Krishnan and John 1974) showed it to be sexually dimorphic, with females producing up to seven broods over a period of 50 days. Furthermore, these authors also emphasised the economic importance of *M. zeylanica* as part of the primary diet of many fish species and other crustaceans within the estuarine environment.

1.10 Environmental tolerances

1.10.1 Ammonia

Sewage and animal faeces containing high levels of ammonia contribute substantially to high concentrations within aquatic systems. This results in increased nitrogen levels, which could be highly toxic to aquatic organisms (Camaro and Alonso-Castillo *et al*. 2006). Levels as low as 0.32 mM have been reported to affect the fecundity of the amphipod *Hyalella azteca* (Borgman 1994). Khon *et al*. (1994) reported that ammonia affected the LC_{50} values for the 10 day bioassay using the amphipod *Leptocheirus plumulosus.* Borgman (1994) also reported that ammonia toxicity resulted in continuous mortality of *Hyalella azteca* for up to 10 weeks for both juveniles and adults. It is thus evident that ammonia is highly toxic to amphipods and a potential confounding factor in toxicity tests. Ammonia sensitivity is, therefore, important for toxicity tests

to ensure that results of the bioassay are caused by the stimulant and not ammonia.

Ammonia in solution primarily exists in two forms, un-ionized ammonia (NH_3) and ammonium ion (NH_4^+), the equilibrium of which is highly dependent on temperature and pH. Un-ionidized ammonia is much more toxic to aquatic organisms than ammonium (EPA 2011). Un-ionidized ammonia can permeate into tissue and enter an organism's body if pH in the organism's body is lower than the surrounding environment (Qiao and Li 2005). This study reports on total ammonia concentrations (NH_4^+ and NH_3).

1.10.2 Salinity

Macrobenthos distribution in estuaries is closely related to the tolerance range of different taxa to environmental variables, with some estuarine benthic species being able to utilize a range of estuarine environments due to their wide-ranging tolerances, but others have more limited distributions due to more specific habitat requirements. Salinity is one of the main environmental factors that affect macrobenthic distribution in estuaries, including that of amphipods (Delgado *et al*. 2011). The salinity tolerance of toxicity test organisms is an important variable in determining its suitability in bioassays, as a stenohaline amphipod will have limited potential as a test organism. It is also necessary to understand whether the survival of amphipods in high salinity conditions does not translate negatively to their reproduction and growth. The determination of the potential capacities of a population in relation to salinity conditions is, therefore, an important prerequisite when conducting more complex bioassays.

1.11 Motivation for the study

Although industrial practices have played a commendable role in the development of South Africa's economy, they have a potentially negative impact on the country's estuaries as most industrial effluents end up in rivers and ultimately in estuaries. As it has been stated earlier, estuaries receive nutrients from both marine and freshwater systems and therefore tend to accumulate nutrients. It is important that the status of estuaries be evaluated by environmental managers so as to determine their conservation status and to ensure sustainability and conservation of important ecosystems. Furthermore, fodder organisms such as amphipods support the growth of larger organisms such as fish and prawns within estuarine ecosystems.

According to the literature, South Africa is not at the forefront of international environmental research (Wepener and Degger 2012). Sus *et al*. (2012) reported that the top five active countries in estuarine bioassays are the US, China, UK, Germany and France. It was noted in

this overview, however, that Australia has also produced a considerable number of papers in the past 10 years. There is, however, a need for more work on toxicity in this field to be conducted in Africa. Zinc and Cu have been shown worldwide to be contaminants of concern in many industrial areas and estuaries (Classon *et al.* 2004; King *et al.* 2006a; Manyin and Row 2006; Hook *et al*. 2014). For this reason, this study focused on the toxicity of Cu and Zn in an estuarine environment using the amphipod *M. zeylanica*. Determining amphipod metal bioaccumulation will not only alert us of the toxicity status of ecosystems, but it will also provide essential information on food chain transfer and eventually biomagnification potential (McPherson and Chapman 2002) as well as enabling development of standard toxicity tests for estuarine areas in South Africa.

Estuarine toxicity is one of the major challenges facing South Africa as industrial development increasingly affects the rapidly diminishing natural estuarine habitat. South Africa is a semi-arid country with a limited and largely ephemeral water supply, and most freshwater resources are already optimally utilized. Furthermore, South Africa is a developing country with limited funds, and the cost of environmental research is thus an important issue. It is, therefore, essential that toxicity tests be developed that are relevant to environmental decision making and easy to apply as well as financially affordable. The challenge is to ensure that even though the country is rapidly developing, development in ecosystems remains sustainable such that aquatic organism diversity is effectively conserved.

This study aimed to test the sensitivity of the amphipod *M. zeylanica* to Cu and Zn in acute and chronic sediment toxicity tests in order to evaluate its suitability as a benchmark test species for toxicity evaluation in South African estuaries. Furthermore, its sensitivity to contaminated estuarine sediments was assessed and validated using Richards Bay Harbour sediment from potentially contaminated sites.
The first part of the study comprised a literature review of the development of chronic sediment toxicity tests using amphipods, with specific reference to Cu and Zn toxicity.

1.12 Hypotheses

1. *Melita zeylanica* is suitable for acute and chronic toxicity testing using Zn and Cu in subtropical estuaries.
2. *Melita zeylanica* is tolerant to ammonia.
3. *Melita zeylanica* is tolerant to salinity ranging between 0-40.
4. *Melita zeylanica* is sensitive to sediment metal concentrations in contaminated sediment

from Richards Bay Harbour.

5. *Melita zeylanica* is a suitable bioindicator species for sub-tropical estuarine systems in South Africa.

1.13 Objectives:

To assess the development of chronic sediment toxicity tests using amphipods, with specific reference to Cu and Zn toxicity, through a review of the relevant scientific literature.

To determine LC_{50} values for *M. zeylanica* when exposed to Zn and Cu during 10 day acute sediment toxicity exposures.

To determine EC_{50} values for *M. zeylanica* when exposed to Zn and Cu during 28 day chronic sediment toxicity exposures, using growth and fecundity as endpoints.

To assess the suitability of *M. zeylanica* as a bioindicator of Cu and Zn toxicity in estuarine sediment using acute and chronic sediment toxicity tests.

To investigate the sensitivity of *M. zeylanica* to ecologically relevant ammonia concentrations and salinities using 96hr water only exposure.

To validate the suitability of *M. zeylanica* as a bioindicator of sediment metal contamination in Richards Bay Harbour using sediment sampled from contaminated and uncontaminated areas.

To determine heavy metal concentrations in sediment from contaminated and uncontaminated areas in the Richards Bay Harbour.

CHAPTER 2

OVERVIEW OF AMPHIPOD SEDIMENT TOXICITY BIOASSAY DEVELOPMENT DURING THE PAST 10 YEARS

(2003-2013)

2.1 Introduction

Metals tend to settle on and accumulate within estuarine sediment, it is for this reason that estuarine sediments are referred to as a metal sink. Benthic organisms, such as amphipods, therefore tend to be inevitably exposed to these metals as they are in direct contact with the sediment (Chapman 1995). According to a report by McDonald *et al*. (2010), some of these metals can cause adverse effects such as changes in benthic community structure.

Sediment toxicity testing has increasingly been reported on over the past 10 years as it was realized that changes in sediment metal concentrations are not as frequent as in water bodies and that sediment analysis allows long-term evaluation as well as integrate, in time and space, the pollution of a given water body (Smith and Owens 2014).

The aim of this component of the study was to conduct a literature review on Cu and Zn estuarine sediment toxicity in amphipods using the EPA, Science Direct and ISI Web of Knowledge databases, focusing on their sensitivity to Cu and Zn. Copper and Zn were chosen because they are both essential elements and therefore are required by amphipods in low concentrations, however, they can be harmful in high concentrations. Furthermore, Cu and Zn are amongst the most frequently reported contaminants in estuarine toxicity testing, thus information on these two metals is more readily available. This overview included literature on bioassays from 2003-2013.

2.2 Objectives

To determine the number of Cu and Zn toxicity studies in amphipods (field and laboratory) in the literature based on EPA, Science Direct and ISI Web of Knowledge databases.

To investigate the frequency of use for various toxicity endpoints (i.e. behaviour, bioaccumulation, growth and reproduction).

To determine the amphipod species most used in toxicity testing and why.

2.3 Materials and Methods

Information was assembled through the EPA, Science Direct and ISI Web of Knowledge databases, using peer reviewed articles published from 2003 to 2013 on the effect of Cu and Zn toxicity on estuarine sediment dwelling amphipods and on the endpoints used in toxicity testing

i.e. LC_{50}, EC_{50}, growth and reproduction. Sensitivity to Zn and Cu were compared and conclusions based on accumulation, reproduction (EC_{50}), growth and bioaccumulation reported.

2.4 Results:

A total of 108 publications were obtained on Cu and Zn bioassays using amphipods. As evidenced in Fig. 2.1, the majority were chronic bioassays, suggesting that there have been a substantial number of chronic toxicity bioassays over the past 10 years.

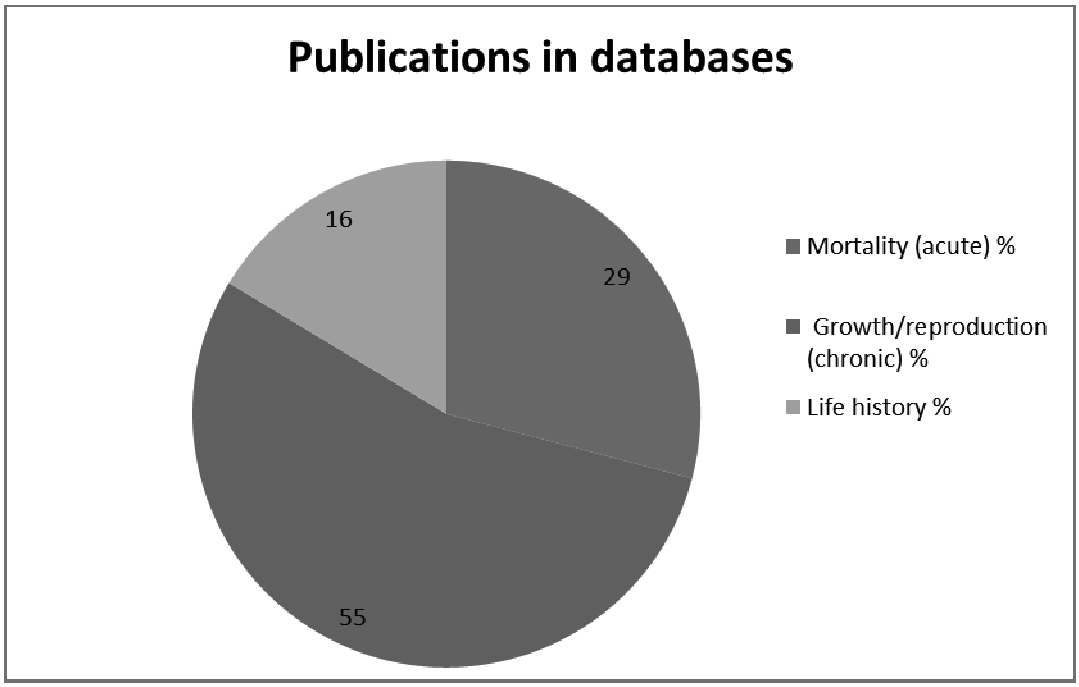

Figure 2. 1 Results on publications of three topics from the overview study.

It was evident that sub-lethal chronic tests did not necessarily imply an extended period of exposure, as one of the chronic bioassays only lasted for a relatively short period (130 minutes). Table 2.1 shows that accumulation and fecundity warranted the longest bioassay time periods, with behaviour mostly conducted in shorter periods. Some bioassays, however, lasted for periods of up to 18 months. The most reported bioassay durations were 30, 28 and 10 day, respectively. Even though the 10 day bioassay studies are still relatively frequently reported, longer duration exposure bioassays have increasingly been conducted. These results, therefore, suggest a transition in toxicity bioassays towards chronic bioassays, which provide more time integrated results, thus they reflect actual environmental concentrations better.

Even though the results of this study suggest that there have been numerous amphipod species used in bioassays within the past 10 years, it is still evident that *C. volutator* has continued to be the amphipod of choice for bioassays. This could be attributed to it being an easily accessible, intertidal amphipod living on estuarine mudflats in Europe and USA and by far most toxicity work was done in these areas (Kahle and Zauke 2003; Classon *et al.* 2004; Costa *et al.* 2005; Rainbow 2006).

It was interesting to note that gammerid amphipods were the most commonly used amphipods in different countries (Table 2.2). Tube dwelling, epibenthic, as well as burrowing amphipods were all used and there does not seem to be a preferred one amongst the three. This, however, could mainly be a factor of availability as well as abundance rather than the preferred choice of amphipods. It has been previously reported that burrowing amphipods are preferred as they are most directly in contact with the sediment (Costa *et al.* 2005).

Table 2.*2* shows that *Melita spp.* is also amongst the sensitive organisms used in bioassay. *Melita plumulosa* was a regular feature in bioassays, one of the unique characteristics of *M. plumulosa* is that it is inhabits both estuarine and freshwaters and, therefore, has high tolerance for physico-chemical parameters, while being sensitive to metal contamination (Lowry *et al.* 2000a; Lowry *et al*. 2000b; Lowry 2004).

Corophium colo showed the highest LOEC value for both Zn and Cu (Table 2.2), this amphipod was also reported to be the least sensitive by King *et al.* (2006b). King *et al*. (2006b) also reported that, with the exception of *M. matilda,* the *Melita spp.* were amongst the most sensitive species with LC_{50} values below 200 µg/g for Cu, which is remarkably low when compared to some amphipods such as *Leptocheirus plumulosa* with LC_{50} values of 886 and 763 µg/g for Cu and Zn, respectively (Table 2.2).

The most frequently used endpoint in chronic toxicity bioassays was fecundity and *C. volutator* was the most frequently used amphipod in chronic toxicity bioassays over the past 10 years, this, however, was found to be a factor of abundance rather than preferred choice of amphipod. The overview, therefore, shows a relatively high percentage of chronic publications (55%) as opposed to acute and life history tests (Fig. 2.1). This result is thus contrary to previous concern that acute amphipods are more extensively reported as compared to chronic tests (Costa *et al.* 2005). More work, however, still needs to be conducted with regards to amphipod life-history bioassays including the entire life-cycle of amphipods.

Table 2. 1 Amphipod species used in chronic bioassays with end-points and exposure period indicated.

Species used	Behaviour	Bioaccumulation	Fecundity	Growth	Exposure period
Anonyx sarsi			*		28d
Chaetocorophium lucasi			*		30d
Chaetocorophium lucasi		*			30d
Chaetocorophium lucasi		*		*	30d
Chaetogammus marinus		*			26d
Corophium colo			*		18 months
Corophium orientale		*			10 day
Corophium volutator	*				130 min
Corophium volutator	*				96hr
Corophium volutator	*				10day
Corophium volutator			*	*	100day
Eogammarus confervicolus			*	*	18 months
Grandidierella japonica			*	*	18 months
Gammarus pulex		*			96hr
Gammarus pulex	*				96hr
Hyale crassicornis			*		30day
Hyale longicornis			*		30day
Hyalella azteca	*				7day
Hyalella azteca		*	*	*	14day

Hyalella azteca		*			17day
Hyalella azteca		*	*	*	28day
Leptocheirus plumulosus		*			10day
Leptocheirus plumulosus			*	*	42day
Melita awa			*		30day
Melita insidiosum				*	96hr
Melita matilda			*		30d
Melita plumulosa				*	48h
Melita plumulosa		*			10d, LOEC
Melita plumulosa			*		10day
Melita plumulosa	*				11day
Melita plumulosa			*		14day
Melita plumulosa			*		30day
Orchestia gammarellus		*			17day
Orchestia gammarellus		*			10day
Orchomeme pelbs		*			10day
Orchomenella minuta			*		28day
Orchomenella pinguis			*		28day
Psammonyx terranovae			*		28day

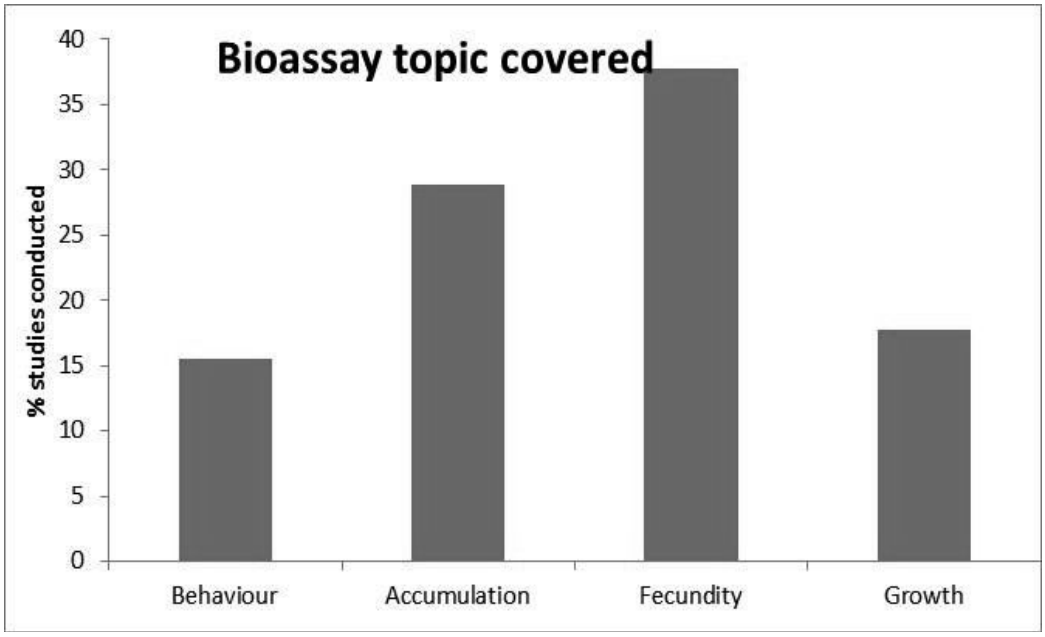

Figure 2. 2 Fecundity of bioassay end-points for chronic toxicity testing based on studies published between 2003 and 2013.

Of the 108 publications obtained, the most reported amphipod was found to be *Corophium volutator* (14%), followed by *Melita plumulosa* (12%) (Fig 2.3). The most reported reason for choice of organism was abundance, however, ease of handling organisms in the laboratory, sensitivity and species distribution were also among some of the reported reasons for choice of organism.

Most chronic bioassay studies comprised 10, 28 or 30 day bioassays (Fig 2.4). The exposure period ranged from 130 min up to 18 months. It was also evident that while longer duration bioassays involved sub-lethal endpoints such as growth and fecundity, shorter duration exposures (130 min to 96 hrs) reported on behaviour, while intermediate exposures (7 to 14 days) reported on bioaccumulation. Acute bioassays were excluded from further analysis in this overview as the main focus of the study was to report on chronic toxicity studies. All studies discussed further are thus chronic studies. This result is contrary to the general understanding that shorter duration bioassays are acute studies.

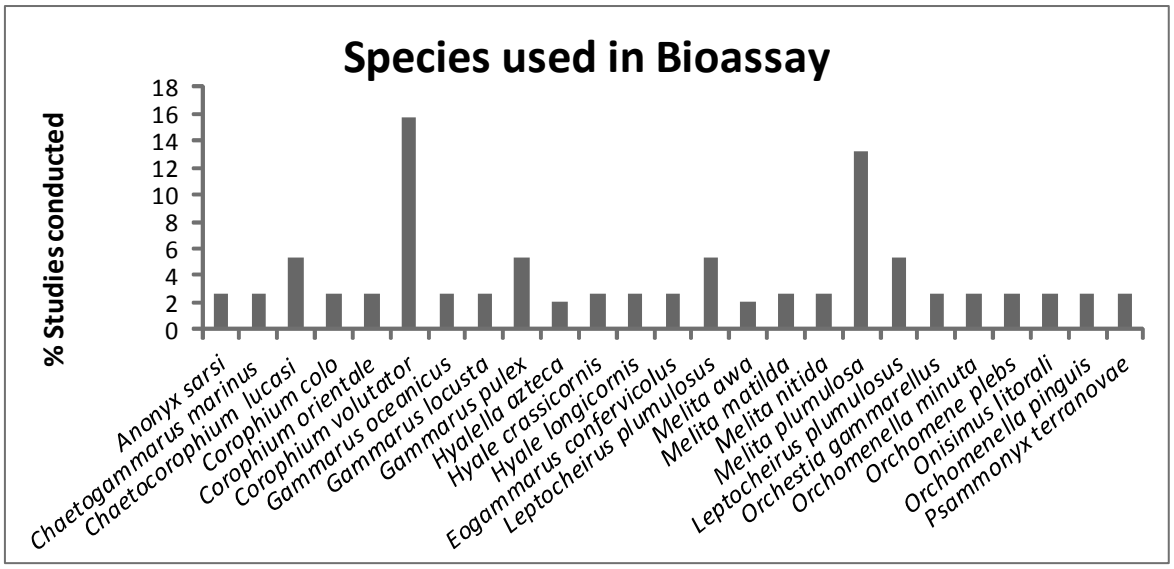

Figure 2. 3 Amphipod species used in chronic bioassays based on studies published between 2003 and 2013.

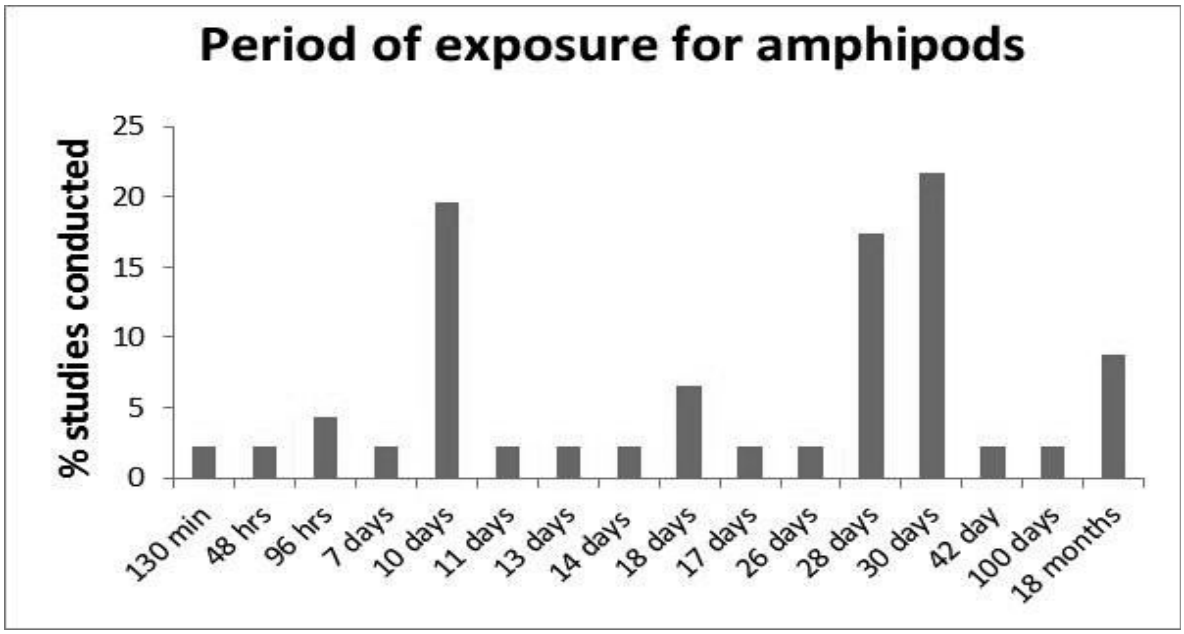

Figure 2. 4 Duration of bioassays exposures reported on studies published between 2003 and 2013.

Table 2.2 shows some of the most active authors and the countries in which they performed amphipod toxicity bioassays over the past 10 years (2003-2013). The amphipod species they worked with, and the conditions under which bioassays were conducted, and metal concentrations recorded are covered in detail in the discussion.

Table 2[LV1]. 2 Comparison of metal concentrations of different amphipods from various bioassays showing lowest and highest accumulations as well as physico-chemical parameters.

Environmental Condition	outcome (values in µg.g-1 d.w.*)	species	Author	Area and type of amphipod
salinity 17, temperature 20	LC 50 Cu 886 ug.g-1, Zn 763 ug.g-1	*L. plumulosus*	Hannah et al. 2013,	United states
in situ	bioconcentration Zn: 670-2400, Cu: 1700-3800	*Paramoera walkeri*	Classon et al. 2003	Gammarid, epibenthic. UK
salinity 32, temperature 11	*LC 50 Cu:37.59 (26.07–54.20), Zn: 14.12 (10.03–19.85)ug.g-1*	C. volutator	Batt and Raffaeli 1998	Gammarid, burrowing, UK
salinity 33.5–34.5, temperature : 25	bioaccumulation 4030 (Cu), 5210 (Zn)	*Orchomene plebs*	Kahle and Zauke, 2003	Gammarid, Germany
salinity 35, temperature: 10	bioaccumulation Cu, 251–520	*Gammarus oceanicus*	Classon et al 2004	Gammarid, Germany
Salinity 13, temperature 25	bioaccumulation Cu, 67.5-93.1, Zn: 80.2-108	*L. plumulosus*	Hannah et al. 2013	Gammarid, United States
in situ	bioaccumulation Cu:4-106, Zn:33-1072	*C. volutator*	Rafaeli & Marr 1998	Gammarid, UK
Salinity 30, temperature 20	bioaccumulation Cu LOEC 820, Zn 2290	*M. plumulosus*	Hook et al. 2014	Gammarid, epibenthic, Australia
Salinity 33, temperature 10	Bioacuumulation Cu 38–59	*Chaetogammarus marinus*	Classon et al 2004,	Gammarid, epibenthic, UK
Salinity 33, temperature 10	Bioaccumulation Cu: 251–520	*Gammarus oceanicus* Segerstråle	Classon et al 2004	Gammarid,eulittoral, Norway
Salinity 33, temperature 20	Bioaccumulation Cu: 70, Zn 78	*Gammarus locusta*	Neuparth et al. 2005	Gammarid, Portugal
Salinity 30, temperature 21	Bioaccumulation Cu: 361, Zn: 221	*Chaetocorophium* cf. *lucasi*	King et al. 2006	tube dwelling, Australia
Salinity 30, temperature 21	LC 50 Cu: >950, Zn: >4500	*Corophium colo*	King et al. 2006	tube dwelling, Australia
Salinity 30, temperature 21	LC 50 Cu: 250, Zn: 1560	*Grandidierella japonica*	King et al. 2006	tube dwelling, Australia
Salinity 30, temperature 21	LC 50 Cu: not reported for adults but >100 for Juveniles	*Hyale crassicornis*	King et al. 2006	epibenthic, Australia
Salinity 30, temperature 21	LC 50 Cu:>190, Zn: 1940	*Hyale longicornis*	King et al. 2006	epibenthic, Australia
Salinity 30, temperature 21	LC 50 Cu: 150, Zn: 710	*Melita awa*	King et al. 2006	epibenthic, Australia
Salinity 30, temperature 21	LC 50 Cu: 220, Zn: 730	*Melita matilda*	King et al. 2006	epibenthic, Australia
Salinity 30, temperature 21	LC 50 Cu: 180, Zn 900	*Melita plumulosa*	King et al. 2006	epibenthic, Australia
Salinity 13, temperature 25	Bioaccumulation: Cu 93.1, Zn: 108.0	*Leptocheirus plumulosus*	Rowe & Manyin 2006	Burrowing, US
Temperature 23	Bioaccumulation Zn: 3087	*Hyalella azteca*	Nguyen et al, 2012	Burrowing, Portugal
in situ	Bioaccumulation Cu: 26.6–42.0	*Caprella dilatata*	Guerra-Gracia et al. 2010	Caprellids, Spain
in situ	Bioaccumulation Cu: nd–70.5	*Caprella equilibra*	Guerra-Gracia et al. 2010	Caprellids, Spain
in situ	Bioaccumulation Cu: nd–46.0	*Caprella grandimana*	Guerra-Gracia et al. 2010	Caprellids, Spain
in situ	Bioaccumulation Cu: nd–22.3	*Caprella iparotensis*	Guerra-Gracia et al. 2010	Caprellids, Spain
in situ	Bioaccumulation Cu: nd–427.9	*Caprella penantis*	Guerra-Gracia et al. 2010	Caprellids, Spain
in situ	Bioaccumulation Cu: 60.7–61.3	*Ampithoe* sp	Guerra-Gracia et al. 2010	Gammarids, Spain
in situ	Bioaccumulation Cu: 90.1–91.5	*Apherusa* sp.	Guerra-Gracia et al. 2010	Gammarids, Spain
in situ	Bioaccumulation Cu: 17.6–18.5	*Hyale perieri*	Guerra-Gracia et al. 2010	Gammarids, Spain
in situ	Bioaccumulation Cu: nd–51.8	*H. schmidtii*	Guerra-Gracia et al. 2010	Gammarids, Spain
in situ	Bioaccumulation Cu: 46.8–113.2	*Jassa marmorata*	Guerra-Gracia et al. 2010	Gammarids, Spain

2.5 Discussion

Amphipods are abundantly widespread and can be found in habitats from the abyssal depths, in marine, estuarine, freshwater as well as groundwater environments. Most amphipods are small free-living benthic gammaridians and they tend to dominate the communities in which they occupy as they generally occur in high densities (Conradi *et al.* 1997; Vetter 1998; Poore and Steinberg 1999; Cunha *et al.* 2000). Furthermore, amphipods form a primary food source for predatory fish and birds (Beare and Moore 1997; Bocher *et al.* 2001; Dauby *et al.* 2001) and therefore can potentially transfer accumulated metals along aquatic food chains (Wang 2002).

Organisms tend to accumulate metals in their bodies from their environment. If the surrounding concentrations are high, metals can be accumulated to relatively high concentrations. According to Rainbow (1998), however, a high concentration of a particular metal in one crustacean species may be low in another; therefore, the comparison of relative concentrations in aquatic invertebrates should ideally be intraspecific rather than between families. Amphipods are often chosen as trace metal biomonitors as they are net accumulators of metals and they also conform to most requirements for biomonitor organisms including being relatively sedentary, abundant, easy to identify and resistant to handling stress (Rainbow *et al.* 1993). Accumulated metal concentrations in invertebrates can also potentially provide valuable information in terms of the geographical and temporal variation in the bioavailabilities of toxic metals in aquatic systems (Rainbow *et al.* 2009). Furthermore, as previously mentioned, amphipods do form the primary food source for many fish and other aquatic organisms (Phillips and Rainbow 1994). Amphipods can, therefore, be classified amongst the most suitable organisms for use in toxicity bioassays. Toxicity bioassays are essential in that they assist in evaluating environmental contamination status, particularly in anthropogenically impacted environments such as harbours and estuaries. It is therefore, important that bioassays be conducted worldwide to enable comparison of different environments, more so, environments with similar industrial activities such as harbours and estuaries.

There are however, concerns regarding inter-laboratory comparison of bioassays, including the difference in bioassay methodologies used. Even though most studies based their methodology on recommended guidelines (e.g. USEPA 1994), they also reported some modifications for convenience purposes. Furthermore, there are various environmental and other factors affecting species sensitivity and these could make it even more difficult to compare inter-laboratory

studies. Amongst these factors are; organism weight, salinity, adaptation, grain size, feeding and gender (Bryan and Langston 1992; Luoma and Fisher 1997).

i. Weight

Accumulation is influenced by factors that affect uptake as well as those affecting excretion. If accumulation is expressed in terms of concentration (µg/g), as opposed to content, accumulation is also affected by weight changes of amphipods. In this study, only adult amphipods were used, which helped minimise the impact body size may have on the results.

ii. Salinity

Decreases in salinity are associated with increases in metal uptake rate (Rainbow *et al*. 1993; Rainbow and Kwan 1995). This overview revealed that most studies conducted bioassays with salinity ranges between 30 and 25. There was not much variation between exposures in terms of salinity between bioassays. Some studies were, however, conducted at salinities of 13 and 35 (Table 2.2).

iii. Adaptation

Long term exposure of amphipods to elevated concentrations of bioavailable trace metals may imply that amphipods have evolved biochemical and physiological mechanisms to reduce the potentially harmful effects of the toxicants (Bryan 1976). Tolerant organisms may lead to an underestimation of toxicity in laboratory bioassays, in fact (Luoma 1997) emphasised that the presence of a metal tolerant population of an organism in a particular location may be considered evidence of the ecotoxicological impact in a given environment. It is, thus important to state the sediment metal concentrations of the area of collection to allow a more relevant comparison.

iv. Grain size

Bryan and Langston (1992) and Luoma and Fisher (1997) also stress that toxicity is affected by sediment grain size, particularly covarying with sediment organic content. It is also important to note, however, that *in situ* sediment is most suitable as the amphipods are already adapted to the sediment characteristics and technically it should not affect bioassay outcomes. Sediment grain-size, however, remains an important factor as finer sediments tend to adsorb more metal (Costa *et al*. 2005).

v. Feeding

Different amphipods adopt different feeding mechanisms (filter feeding, herbivory, predation, scavenging and deposit feeding) as well as specific food preferences (Poore and Steinberg 1999; Pennings *et al.* 2000). It is, therefore, possible for amphipods to accumulate metals differently via food assimilation, for example tube dwelling amphipods, may have different feeding strategies as compared to epibenthic or benthic organisms. Furthermore, some amphipods may be omnivores/predators (phoxocephalids) (Oakden *et al.* 1984), while others are detritrivores (*M. zeylanica*) (Krishnan and John 1974). Some organisms may simply prefer different feeding mechanisms e.g. Corophioid amphipods, which either feed inside their tubes using pleopod induced tube-currents or feed outside their tubes using external water currents (Dixon and Moore 1997).

vi. Gender

More studies are increasingly recognising the difference in metal assimilation and accumulation patterns between males and females. While this may be true for some organisms, Burgos and Rainbow (1998) found no significant differences between males and females in *C. volutator*, the most frequently used amphipod in toxicity testing. Burgos and Rainbow (1998) reported similar patterns in assimilation and accumulation in both male and females for Zn, Cd and Co. Gender was not considered in this study, however, based on the results by Burgos and Rainbow (1998), it was concluded that gender is not a significant factor for the purpose of this overview.

2.6 Main findings from the overview

As evidenced in Fig. 2.1, chronic bioassays are most frequently reported. This implies that there has been an increasing shift from acute to chronic bioassays, as according to Rainbow (1998), due to their convenience, acute tests were the more frequently reported in the past. Rainbow (1998) attributed this to the fact that acute tests are faster to complete and relatively cheaper than chronic tests. Chronic exposures, however, are more valuable as they integrate time of exposure thus providing a more realistic indication of environmental contamination (Conradi *et al*. 1997).

When considering chronic bioassays, fecundity was the most frequently reported of all chronic tests over the past 10 years, with bioaccumulation being the second highest (Fig 2.2). This could be due to the fact that fecundity is the easiest to measure as it can be conducted during

the running of the experiment, while growth and accumulation can only be measured at the end of the experiment. Behaviour on the other hand, can also be conducted while the experiment is running. However, it is time consuming as it requires continuous observation to ensure that the observed behaviour is a result of the stimulant intended and not because of other external influences. Furthermore, fecundity is most valuable as it more accurately reflects the health of individual organisms and the populations, i.e. when organisms are stressed they are likely to channel their energies to survival and growth before reproduction, thus reproduction suffers the most in stressed environments.

CHAPTER 3

STUDY AREA, MATERIALS AND METHODS

3.1 Study area - Richards Bay Harbour

Richards Bay Harbour is a semi-enclosed estuary situated north of KwaZulu-Natal, South Africa, (entrance at 32°02′E, 28°48′S). Richards Bay is characterised by a subtropical climate with warm moist summers at an average of 26^{0}C and moderate winters averaging at 18^{0}C. The estimated mean annual precipitation is at 1 092 mm per annum, summer precipitation is mostly from November to March with maximum precipitation in January and February. Winter rainfall is mostly expected from May to September (www.weathersa.co.za/climate).

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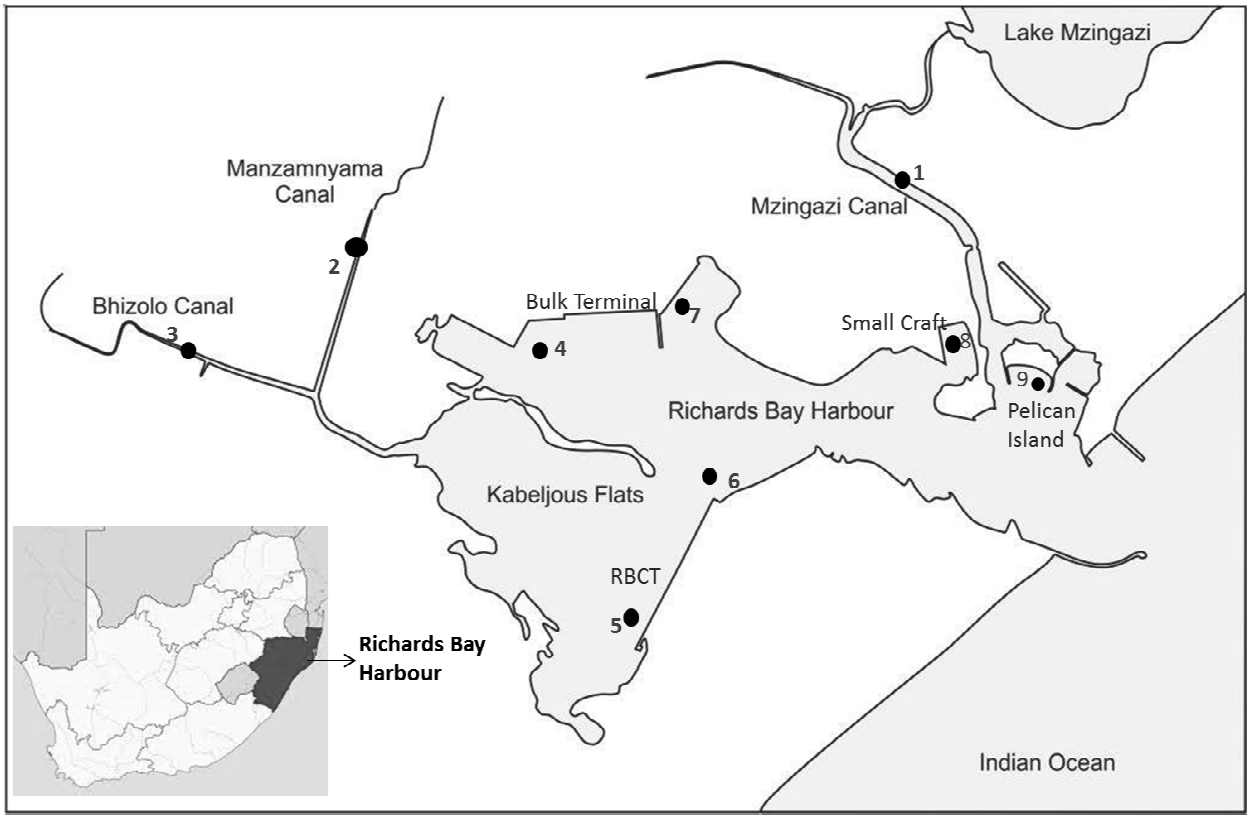

Figure 3. 1 Map of Richards Bay Harbour showing the position of sampling sites.

3.2 Materials and methods

3.2.1 Collection and handling of amphipods

Amphipods were collected from the Mzingazi Canal, which drains into Richards Bay Harbour, using plastic containers (Fig. 3.1). The containers were rinsed using de-chlorinated water and

then filled with estuarine water and a thin layer of sediment collected *in situ.* Amphipods were gently washed from the underside of the rocks which they inhabit. Containers with amphipods were immediately transported to the laboratory where *M. zeylanica* were separated from other amphipods. Amphipods were maintained at a temperature of 25℃, salinity of 25, pH of 7± 0.2 and ammonia was kept under 0.8 mg/l using a biological filter.

Amphipods were cultured under controlled conditions in climate chambers in aerated culture trays with 12hr daylight length. Artificial sea water (Seachem brand) was used for changing water. Water in culture containers was renewed once a week and the amphipods were fed twice a week with Sera Micron at 0.5 mg of Sera Micron (Sera, Heinsberg, Germany) per amphipod.

3.2.2 Collection and preparation of test sediment

Sediment used in toxicity testing was collected from a subtidal mudflat in the upper Mzingazi Canal, a known uncontaminated area in the Richards Bay Harbour (Wepener and Vermeulen 2005). After collection, the sediment was sieved with a 500 µm mesh stainless steel sieve to remove macrofauna and debris, after which it was stored in acid washed plastic containers at 4 ℃ for a maximum of two weeks prior to use. Sediment was left at room temperature for a minimum of 24 hours prior to use in a toxicity test. The culture success of *M. zeylanica* confirmed that sediment was acceptable to conduct toxicity tests.

The spiking procedure to create the test sediment was based on the method described by Swartz *et al*. (1985), with minor modifications. The metals used in the exposure experiments, Cu and Zn, as CuCl and $ZnCl_2$, were directly added from the stock solution to the control sediment to achieve the required nominal concentrations (expressed on a dry weight basis). This was followed by 30 min of mechanical (stirring by hand) mixing. The sediment was then allowed to equilibrate overnight at 25 ℃ before amphipods were introduced.

3.2.3 28 day chronic toxicity test procedures

28-chronic toxicity tests were conducted using standard protocols with some modifications for the use of local species (Chapman and Wang 2001). A 6 by 5 grid was used to conduct the experiments. A range-finder test was run to determine appropriate concentrations at which amphipods were to be exposed. Amphipods were exposed to five metal concentrations and a control sediment with five replicates per concentration in 250 ml beakers containing 200 ml of test water and 2 cm of homogenised test sediment at nominal concentrations ranging between 1.5-7.5 µg/g for Zn and 0.5-4.5 µg/g for Cu. Water, at a salinity of 25, was gently added to each

beaker, using a plastic spoon to prevent disturbance of the surface sediment. Beakers were covered with plastic petri dish lids to minimize evaporation, each with a small hole drilled through the centre to allow aeration of the test beaker with a glass pipette. Beakers were supplied with a gentle flow of air throughout the tests to maintain dissolved oxygen concentration in the overlying water at >80% saturation. Sediments and overlying water were added to the beakers at least 12 hours prior to the start of a test and placed in an environmental chamber at 25 ℃ to allow the sediment to settle overnight. For the duration of the toxicity test, beakers were kept in the temperature-controlled environmental chamber at 25 ˚C with a 12:12 hour light:dark photoperiod and a constant light intensity (60 W).

At the start of each test, 8 amphipods (5 females and 3 males) were gently released in each beaker using a plastic pipette to avoid injury to the amphipods. After release, the amphipods were carefully monitored for 1hr to check for burrowing activity and any inactive amphipods or ones showing erratic behaviour were replaced. Test beakers were monitored every second day for abnormal behaviour and oxygen flow. For quality assurance purposes, physico-chemical parameters including dissolved oxygen concentration (>80%), ammonia (<0.8 mg/l), pH (7.5–8.2), salinity (25 ± 1) and temperature (25 ± 0.2 ºC) were monitored periodically throughout the test and at test termination to ensure that all variables remained within acceptable limits throughout the test. Ammonia, however, could not be monitored for the entire duration of experiments as the photometer (Merck Spectroquant SQ118) malfunctioned and could not be repaired until the end of experiments. In order to avoid ammonia build-up, the water was changed once a week using artificial seawater and amphipods were fed once a week with Sera Micron at 0.5 mg of Sera Micron per amphipod.

At the end of the 28-day period, amphipods were gently sieved from the sediment and counted, blotted dry, rinsed twice with double distilled water to remove any residual sediment and debris and stored in the freezer for later recording of length as well as metal analysis. Samples of the sediment were collected from all 5 beakers per exposure for metal analysis and stored for later analysis. The length of juvenile amphipods (offspring of adults in the bioassay) was measured using a calibrated stereo light microscope, to determine growth of amphipods.

All glass and plastic-ware used during the toxicity testing were cleaned by soaking for 24 hrs each in phosphate-free detergent and 5% HNO_3 acid diluted with double distilled water, followed by rinsing in double distilled water.

3.2.4 Acute toxicity test procedure

The 10 day acute sediment toxicity test procedure was similar to that of the chronic toxicity test with a few modifications. The acute toxicity test was conducted in 250 ml glass beakers containing 40 g of sediment spiked with nominal concentrations of Zn and Cu, ranging between 4-64 µg/g for Zn and 4-64 µg/g for Cu. Beakers were filled to 200 ml with seawater adjusted to a salinity of 25. Amphipods were not fed throughout the 10 day exposure. The acute toxicity test was terminated after 10 days, and all surviving amphipods were counted and analysed for metal content. Sediment and tissue samples were collected from all replicates, and water physico-chemical parameters were measured at the start and termination of each toxicity test.

3.2.5 Reference toxicity test

Cadmium chloride is a commonly used reference toxicant because it is highly toxic to aquatic organisms and it is also a non-essential metal in amphipods. The sensitivity of *M. zeylanica* to $CdCl_2$ was determined during a 96 hrs static water only test using a 6 x 5 grid. Amphipods were added to aerated beakers containing 200 ml of filtered and diluted seawater at a salinity of 25 ± 1 containing $CdCl_2$ at nominal concentrations ranging between 0.4-6.4 mg/l. At the end of the experiment, amphipods were counted to determine the survival per exposure.

3.2.6 96hr Ammonia test

In the ammonia tolerance test, 10 amphipods per 250 ml beaker were added to beakers containing aerated 200 ml of filtered and diluted seawater at a salinity of 25 ± 1. Amphipods were exposed to ammonia (NH_3), added as NH_4Cl,at nominal concentrations ranging between 1-50 mg/l. Water was not changed during the experiment and amphipods were not fed. Amphipods were observed for two hours following the start of the experiments and non-satisfactory amphipods were removed.

3. 2.7 96hr Salinity test

In the salinity tolerance test, a 6 by 5 grid was used with 10 adult amphipods added to 250 ml beakers containing 200 ml water with 1 cm sediment. Amphipods were exposed to salinities of 0, 10, 20, 30 and 40, with 25 being a control. Oxygen was maintained at >80% and pH at 7 ± 0.2 at a temperature of 25 ˚C.

3.2.8 Field validation

The toxicity test procedure using *in situ* sediment collected from potentially contaminated sites in Richards Bay Harbour was similar to that of the acute test with a few modifications. The upper 5-10 cm sediment was collected at 9 different sites (Fig 3.1) in Richards Bay Harbour using a stainless steel Van Veen grab. Sediment was then stored in acid-washed sampling jars and placed in a freezer upon return to the laboratory. The grab was rinsed twice in site water between the collection of consecutive sediment samples.

Major industrial inputs within the harbour include; a bulk coal storage, fertiliser plant, two large aluminium smelters, woodchip exporting plant and a papermill (Weerts 2002). Sampling sites were selected to reflect different uses of the harbour, based on previously reported sediment metal concentrations (Wepener and Vermeulen 2005).

The toxicity test was conducted in 250 ml glass beakers. Although sediment was collected at 9 different sites, the 6 by 5 grid only accommodates six samples to be tested at once. Six sites were then selected according to the diversity of port activities and industrial influences. The six selected sites were; Pelican Island (control site), Bulk (C7) terminal, Manzimnyama Canal, Bhizolo Canal, Coal Terminal RBCT (6) and Mzingazi Canal. Even though amphipod exposure was only reported for the six above mentioned sites, sediment analysis was still conducted for all nine sites sampled.

Test beakers contained 40 g of sediment from each of the six sites with five replicates per site. Eight amphipods were placed in each beaker; beakers were filled to 200 ml with artificial seawater adjusted to a salinity of 25. For the duration of the test (10 days), beakers were kept in a temperature-controlled environmental chamber at 25°C with a 12:12 hour light:dark photoperiod and a constant light intensity (60W). Amphipods were not fed throughout the exposure. The field validation test was terminated after 10 days, and all surviving amphipods were counted. Sediment and whole amphipod samples were collected from all replicates and stored for later analysis. Water physico-chemical parameters were measured at the start and termination of the toxicity test.

3.3 Metal analysis

3.3.1 Sediment preparation

Sediment samples were dried at 60^0C and checked regularly until dry-weight readings became constant. They were then crushed using a pestle and mortar until homogenised (Stephenson 1998). Digestion was conducted using a Milestone microwave digesting system with internal temperature sensor. The 'Mud with 25% organic' method was selected (Stephenson 1998). The sample was weighed to 0.3 g and 8 ml of HNO_3 and 2 ml of H_2O_2 were added as reagents. The digestion method consisted of eight steps which are:

1) Teflon microwave vessel (TFM) was placed on the balance plate, and the sample was weighed.

2) The TFM was then introduced into the vessel holder.

3) Acids were added, if part of the sample stayed on the inner wall of the TFM vessel, the acid was added drop by drop and the solution was gently swirled to homogenize the sample with acids.

4) The vessel was then closed and the cover tightened.

5) The sensor segment was then inserted into the microwave cavity and connected to the temperature sensor and the microwave program was run to completion (1 hr).

6) The rotor was left for ± 2 hrs to allow the sample to cool to room temperature.

7) The vessel was then opened and the solution transferred into a volumetric flask.

8) The solution was then topped up with 10% nitric acid to make up a 25ml solution, after which the sample was stored until analysed.

3.3.2 Tissue preparation

The same procedure as described for sediment preparation was applied for the tissue and the sample was maintained at 0.3 g. A minimum number of 6 amphipods from each exposure concentration was used to obtain the sample size.

3.3.3 Metal analysis

Metal concentrations in sediment and amphipod tissue were determined using a Varian Ultra mass 700 inductive coupled mass spectrometer (ICP-MS). All samples were analysed for aluminum (Al), arsenic (As), Cd, Cu, chromium (Cr), iron (Fe), lead (Pb), nickel (Ni), mercury (Hg) and Zn. 10 mg/l NH_3 was used as an external standard (a known concentration of 10 mg/l NH_3 was analysed after every 10 samples to ensure that the ICP-MS was functioning optimally) and blank samples containing distilled water were run after every 10^{th} sample as an internal standard and to ensure that the ICP was running efficiently. No certified reference sediment was available as quality control standard at the time of sediment metal analysis, which means that the concentrations of metals in toxicity test sediment and field collected samples, could not be verified. This was taken into account in the interpretation of the results.

3.4 Statistical Analysis

The LC_{50} for metal concentrations in *M. zeylanica* was determined using the EPA Probit Analysis software. Following the test for normality, Kruskal Wallis nonparametric test for >2 means was used to test for differences between mean survival, fecundity, growth and sediment and body metal concentrations. Analysis of covariance (ANCOVA) was preformed to determine the influence of adult mortality on fecundity and juvenile growth during the 28 day chronic tests. Pairwise post-hoc comparisons were performed using Tukey's HSD test to find which means were significantly different from one another. Pearson's Correlations coefficients (R) were determined to evaluate the correlation between survival, fecundity and growth, and tissue and sediment metal concentrations.

CHAPTER 4

RESULTS

4.1 Environmental conditions

The environmental parameters monitored throughout the experiment remained within allowed limits (ASTM 2008) and did not impair the outcome of the toxicity tests (Table 4.1). Ammonia could, however, not be monitored to the end of the experimental work as the photometer malfunctioned and could not be repaired. Water was changed halfway through the 10 day acute tests and twice a week during the 28 day tests to ensure that ammonia levels remained low.

Standardised prescribed methods for sediment bioassays (ASTM 2008) require 1L beakers and 200 g sediments per beaker. These measurements, however, were reduced in this study for convenience, i.e. 250 ml beakers were used instead of 1L and 40 g sediment instead of 200 g. The effect of reducing these measurements was investigated by Ferretti *et al*. (2002), who reported that reducing the size of the beaker as well as sediment quantity did not significantly affect the test outcome compared to the standard prescribed methods. Because the reduced size of the beaker offered convenience in reduced space to run the experiments as well as reduced volumes of sediment to be spiked, the measurements used in this study were miniaturized according to Ferretti *et al*. (2002).

Table 4. 1 Environmental variables measured throughout the toxicity tests.

Variable	Unit	Application limits	10 day Zn	10 day Cu	28 day Zn	28 day Cu
Oxygen saturation	%	>80%	78-80%	78-81%	77-80%	78-81%
pH	-	6.5-8.0	6.9-7.0	6.9-7.0	6.8-7.0	6.9-7.0
Ammonia	Mg/l	0.8	-	-	-	-
Temperature	˚C	25±1	24-26	24-26	24-25	24-25
Salinity	-	25±1	24-26	24-26	24-25	24-25

4.2 96hr Exposures

4.2.1 96hr Cd Reference test

Survival of 100% was recorded in the control following the 96hr water-only exposure, with only 6.25% survival in the 2 mg/l exposure. There were no survivors in the two lowest concentrations (Fig. 4.1). The 96hr water-only Cd LC_{50} of *M. zeylanica* using $CdCl_2$ at 25°C and at a salinity of 25 was 1.176 mg/l (Table 4.2). This value was found comparable to the general sensitivity of estuarine amphipods to cadmium (ASTM 2008). The Kruskal Wallis test showed that there was a significant difference in *M. zeylanica* survival during the 96hr Cd test ($p<0.001$) (Table 4.3). Tukey's *post hoc* test showed significant differences in mean survival between the first three exposures (Table 4.4).

Table 4. 2 Probit results showing LC_{10}, LC_{50} and LC_{90} (mg/l) of *M. zeylanica* following a 96hr water-only toxicity test. Confidence limit of 95 was applied for all LC values.

	LC_{10}	LC_{50}	LC_{90}
Cadmium 96hr test	0.47	1.18	2.98

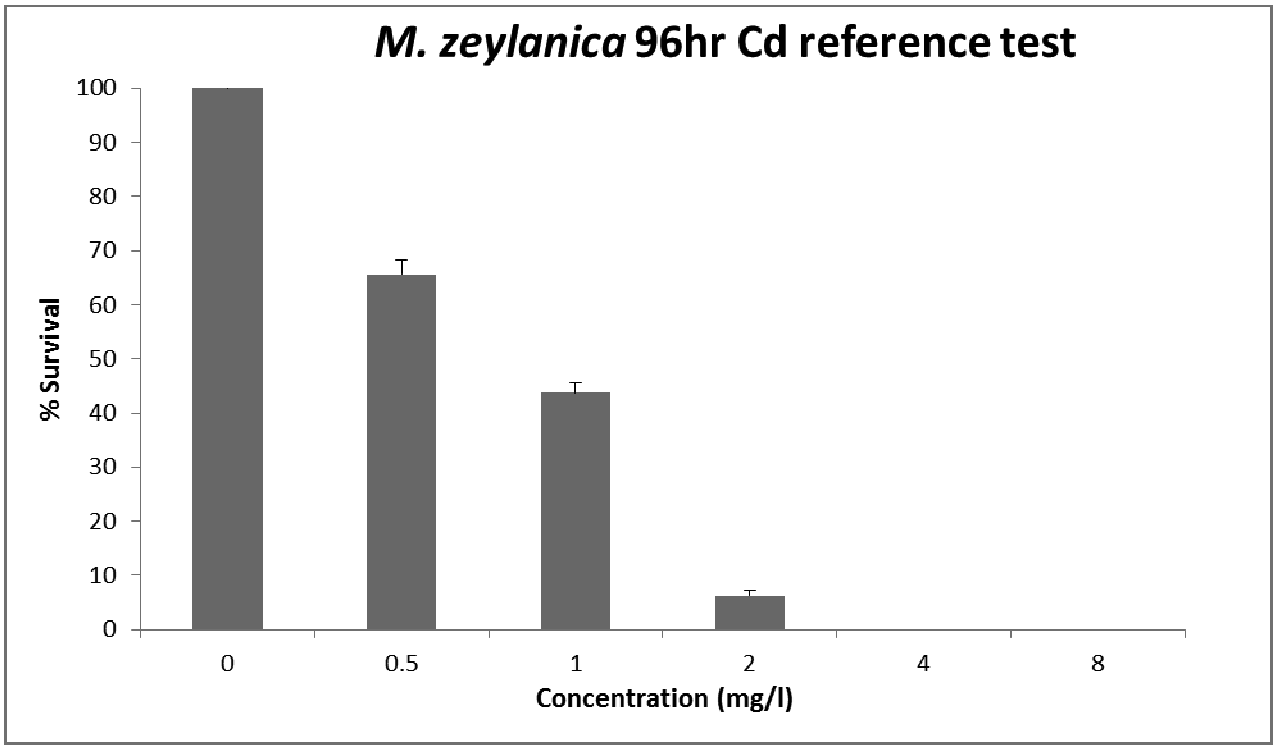

Figure 4. 1 Mean % survival (+STD) of *M. zeylanica* following a 96hr water-only Cd toxicity test with concentrations ranging from 0.5-8 mg/l.

4.2.2 96hr Ammonia tolerance tests

The 96hr ammonia tolerance test indicated 100% survival in the control (Fig. 4.2). The LC_{50} for ammonia was 17.03 mg/l (Table 4.5). *Melita zeylanica* also seemed to tolerate ammonia concentrations up to 10 mg/l quite well, as there was 78% survival in the 10 mg/l exposure. There seems to be ammonia intolerance in *M. zeylanica* above 10 mg/l, with 0% survival at 20 mg/l. The Kruskal Wallis test showed that there was a significant difference in mean survival during the 96hr ammonia exposure ($p<0.001$) (Table 4.3). Turkey's post-hoc analysis showed significant difference in *M. zeylanica* survival between the first three exposures (Table 4.4).

4.2.3 96hr Salinity tolerance test

The 96hr salinity sensitivity test indicated that *M. zeylanica* has a very wide salinity tolerance, with 100% survival at salinities of 10 and 30 and a 98% survival at a salinity of 40 (Fig. 4.3). *Melita zeylanica* was intolerant to freshwater as there was no survival in the freshwater exposure. The reason for the relatively low survival at a salinity of 20 is not known, given that 100% survival was recorded at salinities of 10, 25 and 30. A control salinity of 25 was used as this is the salinity used in the culture trays.

Table 4. 3 Results of Kruskal Wallis nonparametric test for more than two means.

	Chi-Square	df	p
Cd 96hr	22.83	5	<0.001
Ammonia 96hr	46.0	5	<0.001
Zn10 day survival	22.14	5	<0.001
Cu10 day survival	26.76	5	<0.001
Zn 28 day adult survival	7.83	5	0.166
Cu 28 day adult survival	19.36	5	0.002
Zn Fecundity	26.76	5	<0.001
Cu Fecundity	20.40	5	0.001
Field sites	9.69	5	0.85

Table 4. 4 Results of the Post hoc analysis for 10 day sediment and 28 day sediment and fecundity results for Zn and Cu. Exposures designated different letters show significantly different mean survival or fecundity.

Bioassay	Control	1st Exposure	2nd Exposure	3rd Exposure	4th Exposure	5th Exposure
Cd 4 day	a	b	bc	c	c	c
Zn 10 day	a	ab	bc	cd	cd	cd
10 day Cu	a	ab	bc	bcd	bcd	bcd
28 day adult survival Zn	a	a	a	a	a	a
28 day fecundity Zn	a	b	bc	cd	d	d
28 day adult survival Cu	a	a	a	a	b	a
28 day fecundity Cu	a	ab	bc	bc	bc	bc

Table 4. 5 Ammonia tolerance test results, showing the LC_{10}, LC_{50} and LC_{90} (mg/l), for *M. zeylanica* following a 96hr ammonia tests.

	LC_{10}	LC_{50}	LC_{90}
Ammonia 96hr test	10.15	17.03	38.57

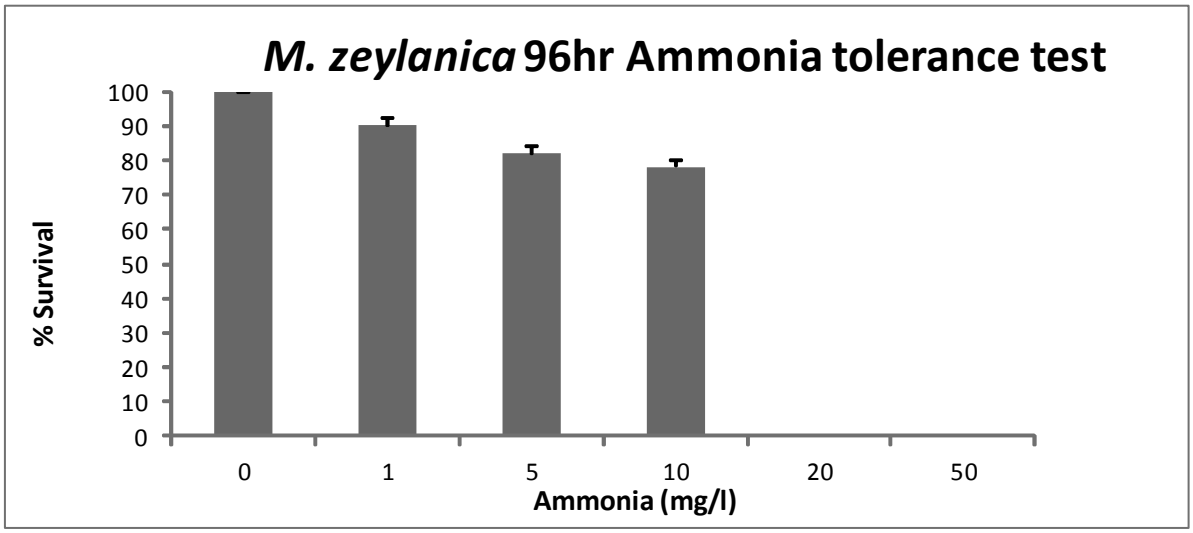

Figure 4. 2 Mean % survival (+1STD) of *M. zeylanica* following a 96hr ammonia tolerance test with concentrations ranging from 0-50 mg/l.

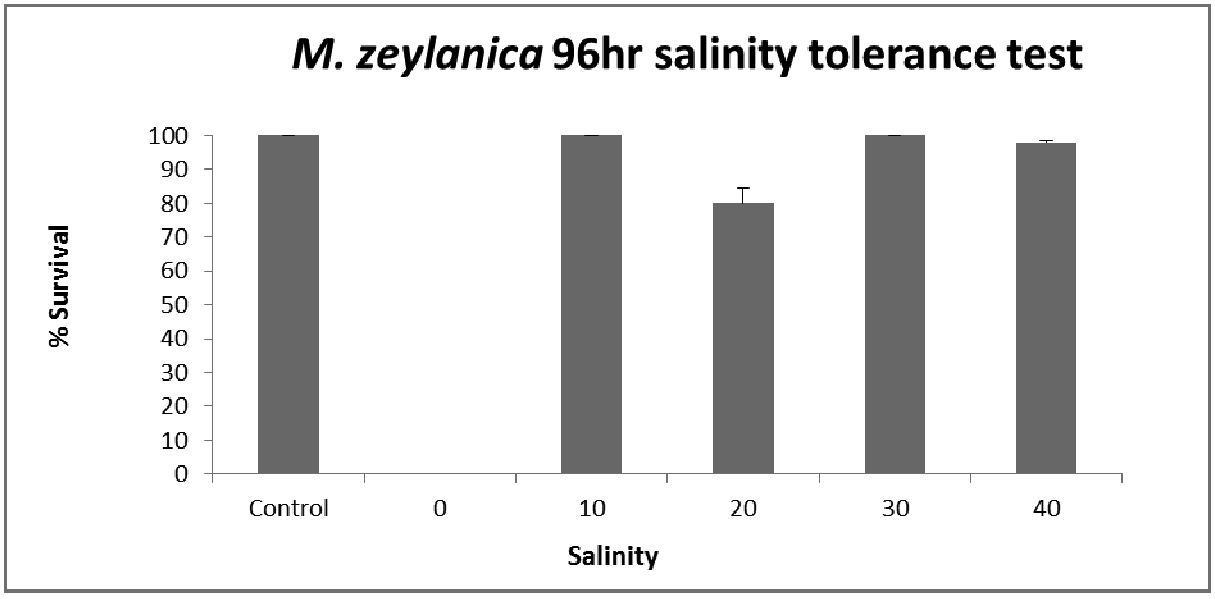

Figure 4. 3 Mean % survival (+STD) of *M. zeylanica* following a 96hr salinity tolerance test at salinities ranging from 0-40 salinity.

4.3 Acute toxicity exposures

4.3.1 10 day Zn acute sediment toxicity test

Following the 10 day exposure of *M. zeylanica* to Zn spiked sediment, mean survival in the control was 100%, demonstrating that the test procedure and water quality were acceptable for conducting a 10 day sediment toxicity test (Swartz *et al.* 1985; ASTM 2008). Amphipod survival

decreased progressively with increasing Zn concentration with the highest concentration showing a 30% survival (Fig. 4.4). The LC_{50} value for *M. zeylanica* exposed to Zn concentrations ranging from 4-64 µg/g was found to be 9.15 µg/g. The LC_{10} and LC_{90} was 0.35 and 238.51 µg/g, respectively (Table 4.6). Although nominal LC_{50} values are reported for all metals in this study, actual LC_{50}'s are higher due to background concentrations, e.g. the 16µg/g exposure is in actual fact (as measured) 103 µg/g. The LC_{50} of 9.15 (nominal) would thus be closer to 100µg/g in the sediment. The Kruskal Wallis test showed there was a significant difference in mean *M. zeylanica* survival between exposures ($p<0.01$) (Table 4.3). Tukey's post hoc test showed significant differences in the mean survival between the first three exposures (Table 4.4).

Table 4. 6 The LC_{10}, LC_{50} and LC_{90} (µg/g) for *M. zeylanica* following a 10 day Zn sediment toxicity test.

	LC_{10}	LC_{50}	LC_{90}
Zn 10 day test	0.35	9.15	238.51

Sediment Zn concentrations increased concurrently with test concentrations (Fig. 4.5, Table 4.7), although Zn concentrations in the sediment were much higher than spiking concentrations, particularly in the 32 and 64 µg/g exposures. Body Zn concentrations in *M. zeylanica* increased with increasing sediment Zn concentrations (Fig. 4.5) (Table 4.7), ranging from 47-252 µg/g, suggesting that *M. zeylanica* accumulated Zn at all exposure concentrations. There was a significant positive correlation between sediment and tissue Zn concentration ($R = 0.91$, $p<0.05$) (Fig. 4.6).

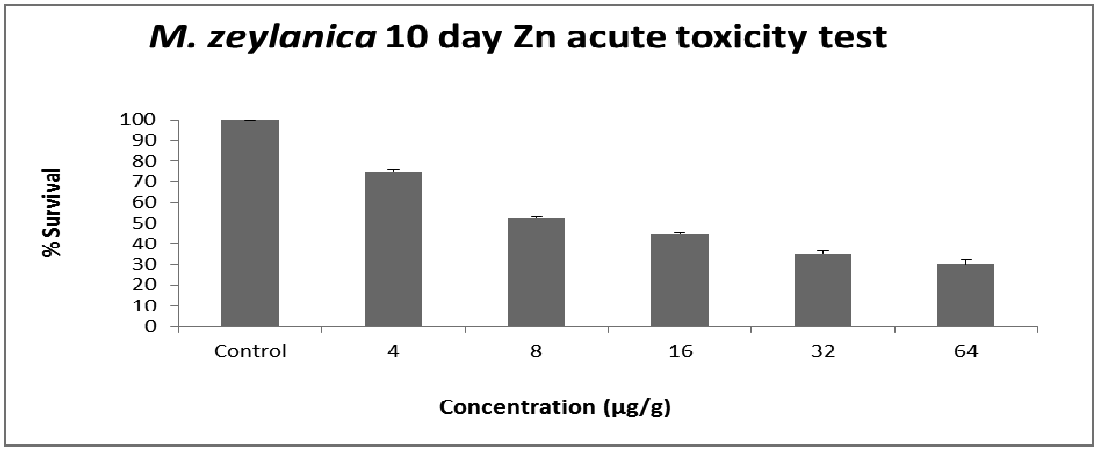

Figure 4. 4 Mean % survival (+1STD) of *M. zeylanica* following the 10 day Zn sediment toxicity test with concentrations ranging from 4-64 µg/g.

Table 4. 7 Mean (+1STD) sediment and tissue Zn concentrations (µg/g) in *M. zeylanica* following a 10 day Zn sediment toxicity test with concentrations ranging from 4-64 µg/g.

Exposure	Sediment	STD	Tissue	STD
Control	50.6	1.4	46.7	0.005
4	58.2	1.2	81.7	0.001
8	90.8	4.8	81.4	0.005
16	103.0	3.2	129.2	0.001
32	234.7	3.9	162.5	0.009
64	236.2	27.9	252.0	0.02

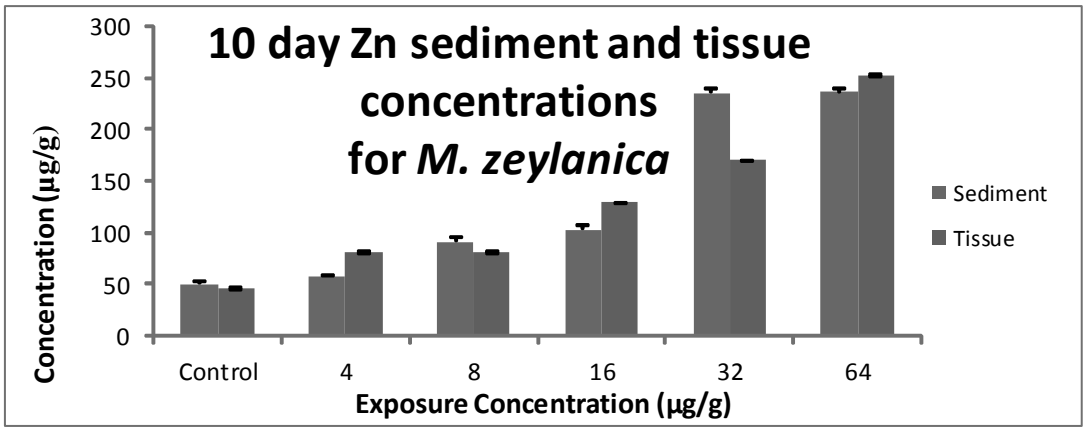

Figure 4. 5 Mean sediment and tissue metal concentrations (+1STD) of *M. zeylanica* following a 10 day sediment toxicity test with Zn concentrations ranging from 4-64 µ/g.

4.3.2 10 day Cu sediment toxicity test

During exposure of *M. zeylanica* to Cu spiked sediment, mean survival in the control was 85% (Fig. 4.7), demonstrating that the test procedures and water quality, were acceptable for conducting the 10 day sediment toxicity test (Swartz *et al*. 1985; ASTM 2008). Amphipod survival decreased progressively with increasing sediment concentration, from 85% in the control to 30% at 32 µg/g. The LC_{50} value for *M. zeylanica* exposed to Cu spiked sediment was 11.8 µg/g (Table. 4.8). Sediment Cu concentrations increased with spiking concentration (Fig. 4.8). Body Cu concentrations also increased with increasing sediment Cu concentrations (Fig. 4.8, Table 4.9). There was a positive correlation between sediment and tissue Cu concentrations (Fig. 4.9).

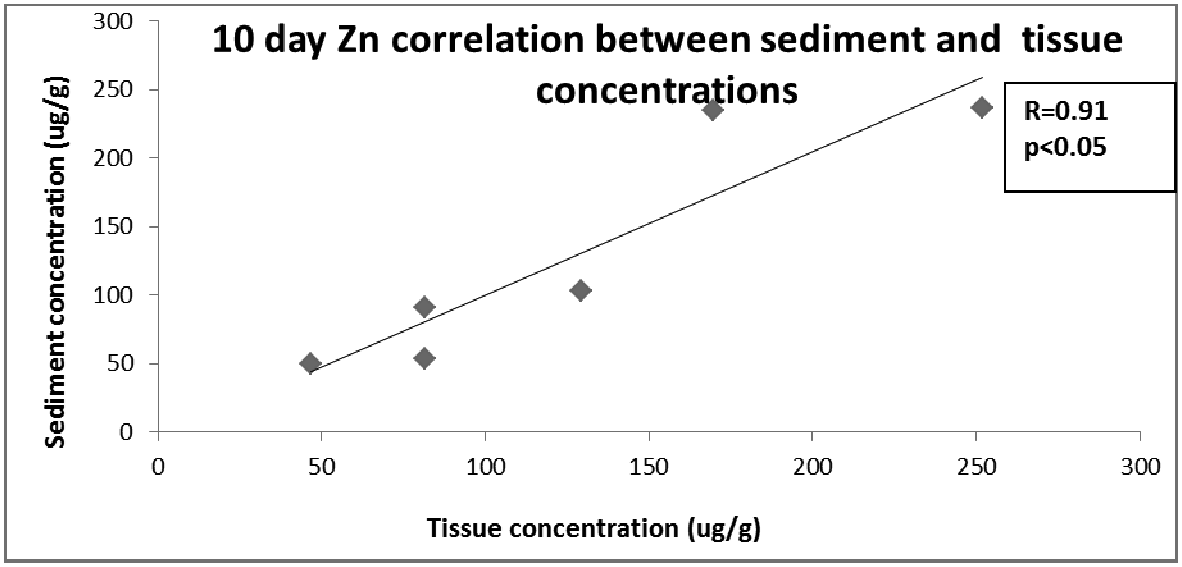

Figure 4. 6 Parson's Correlation between Zn tissue concentrations in *M. zeylanica* and sediment concentrations following a 10 day Zn exposure with concentrations ranging from 4-64 µg/g.

Table 4. 8 The LC_{10}, LC_{50} and LC_{90} for *M. zeylanica* recorded during 10 day Cu sediment toxicity test.

	LC_{10}	LC_{50}	LC_{90}
Cu 10 day Mortality test	1.76	11.76	78.70

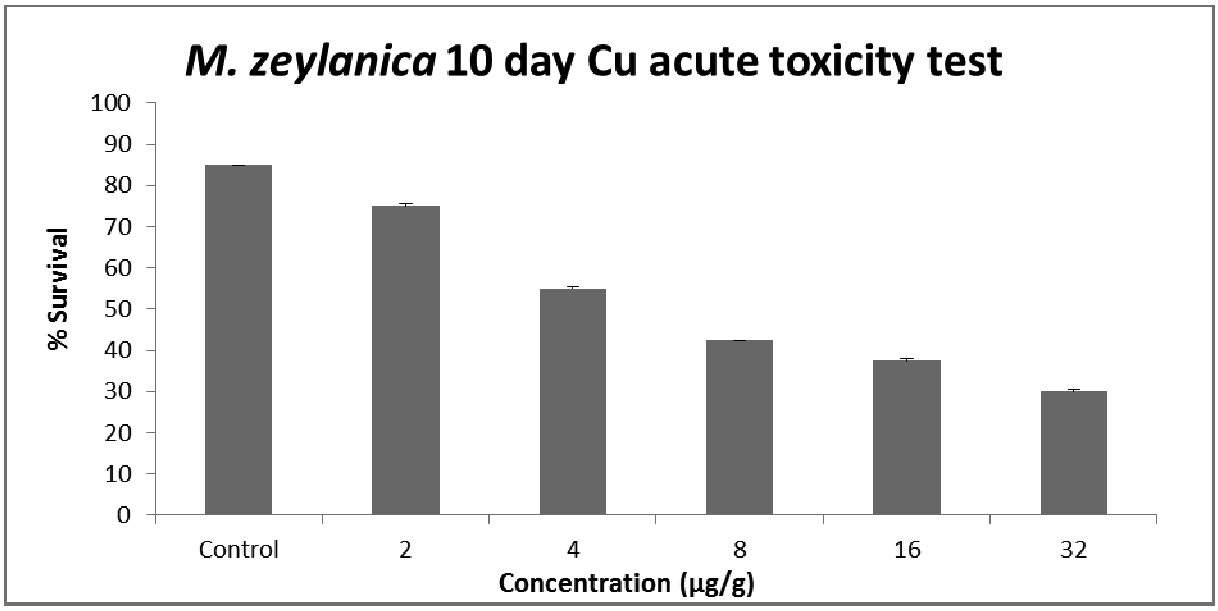

Figure 4. 7 Mean % survival (+1STD) of *M. zeylanica* following a 10 day sediment Cu toxicity test with concentrations ranging from 2-32 µg/g.

Table 4. 9 Mean sediment and tissue Cu concentrations (µg/g) following a 10 day Cu sediment toxicity test with concentrations ranging from 2-32 µg/g.

Exposure	Sediment	STD	Tissue	STD
Control	15.4	*2.7*	51.6	0.3
2	21.2	2.5	50.9	0.9
4	22.5	0.9	70.3	0.8
8	32.9	0.9	80.2	1.9
16	42.7	0.5	100.5	2.3
32	56.3	2.3	92.2	0.2

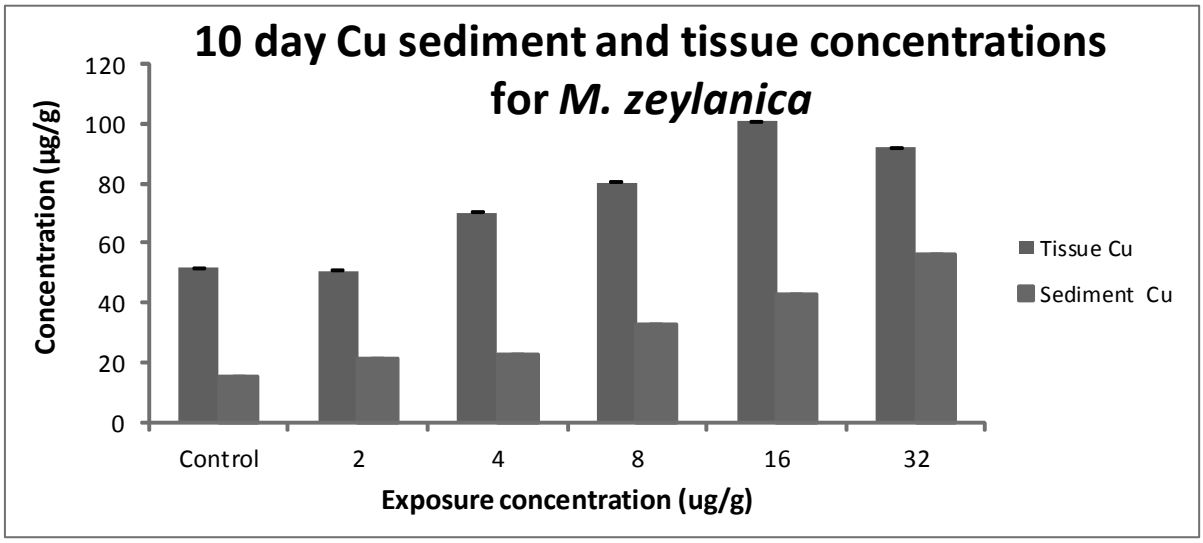

Figure 4. 8 Mean sediment and tissue metal concentrations (+1STD) of *M. zeylanica* following a 10 day sediment toxicity test with Cu concentrations ranging from 2-32 µg/g.

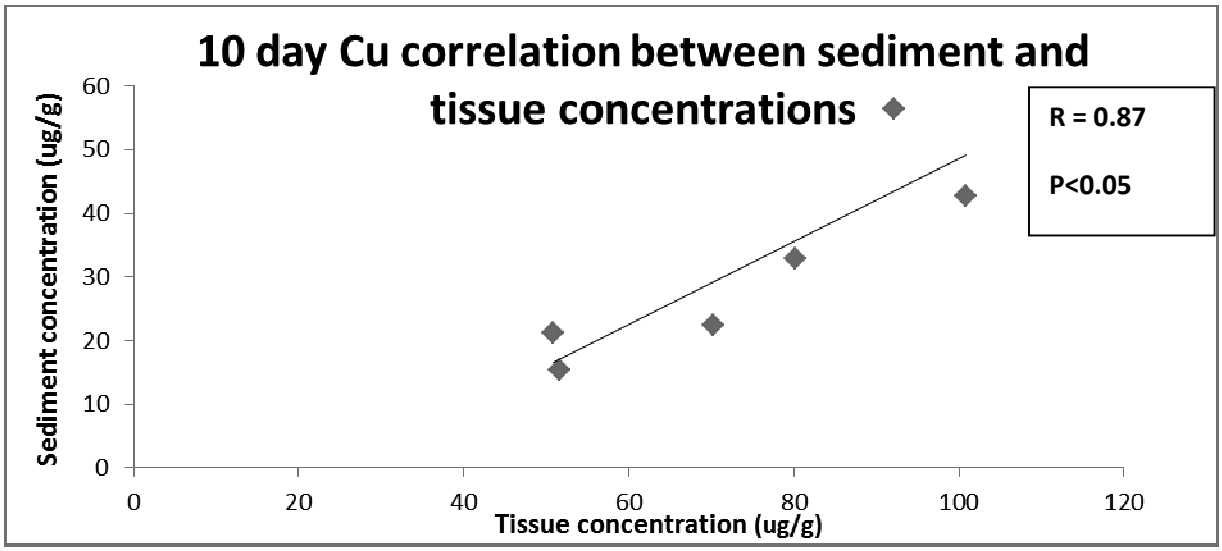

Figure 4. 9 Pearson's Correlation between *M. zeylanica* tissue and sediment Cu concentrations, following a 10 day Zn exposure with concentrations ranging from 2-32 µg/g.

4.4 Chronic toxicity Exposures

Based on the LC_{50} values for Cu and Zn toxicity recorded during the 10 day sediment toxicity tests, relatively low Cu and Zn concentrations were selected for the 28 day chronic sediment toxicity tests. Unfortunately, for the Zn chronic experiment, juvenile growth could not be

accurately determined as, at the time of analysis, a power failure in the laboratory caused the frozen samples to thaw, resulting in the amphipod tissue going soft.

4.4.1 28 day Zn Exposure

During the 28 day Zn exposure, mean adult survival in the control was 87.5% (Fig. 4.10), demonstrating that the test procedures and water quality, were acceptable for conducting the 28 day toxicity test (Swartz *et al.* 1985; ASTM 2008).

Amphipod adult survival decreased slightly with increasing sediment concentration, from 87.5% in the control to 65% at 7.5 µg/g (Fig. 4.10). The EC_{50} value for *M. zeylanica* exposed to Zn sediment concentrations was found to be 2.72 µg/g, while EC_{10} and EC_{90} values were 0.68 and 10.88 µg/g, respectively (Table 4.10). There was no significant difference in adult survival following the 28 day exposure ($p = 0.17$) (Table 4.3). As with the LC_{50}, nominal EC_{50} values are reported, it is therefore important to note that actual EC_{50} values will be significantly higher than the reported actual values. Tukey's *post hoc* analysis showed that there were no significant differences in mean survival for all exposures (Table 4.4).

Table 4. 10 Probit analysis showing EC_{10}, EC_{50} and EC_{90} for *M. zeylanica* following a 28 day fecundity test for Zn.

	EC_{10}	EC_{50}	EC_{90}
Zn 28 day Fecundity test	0.68	2.72	10.89

Table 4. 11 Mean Zn concentration in *M. zeylanica* (µg/g) following a 28 day sediment toxicity test with Zn exposure concentration ranging from 1.5-7.5 µg/g.

	Zn concentration	STD
Control	50.6	1.5
1.5	90.6	3.7
3.0	60.4	1.5
4.5	100.9	2.5
6.0	150.7	3.4
7.5	100.5	1.8

Zinc sediment concentrations increased with increasing exposure concentration, with the lowest sediment concentration being 50.6 µg/g and the highest being 150.7 µg/g (Fig. 4.11) (Table

4.11). Due to the decrease in adult survival during the 28 day test, a co-variance test (ANCOVA) was conducted to determine whether adult survival had a significant effect on fecundity. ANCOVA analysis revealed that adult survival had no significant effect on fecundity ($p>0.05$) (Tables 4.12). Tukey's post hoc test also showed that there was no significant difference in mean survival for all exposures (Table 4.4).

Fecundity decreased progressively with increasing Zn sediment exposure, with the control exposure showing an average of 65 juveniles, while the highest Zn concentration showed an average of only 5 juveniles (Fig. 4.12). There was a significant difference in fecundity between different exposure concentrations, ($p<0.05$) (Table 4.3). *Post hoc* analysis indicated that a significant difference existed between the 1st and 5th exposures and that there were no significant differences amongst other exposures (Table 4.4). Fecundity showed a significant negative correlation ($R = -0.73$, $p <0.05$) with sediment Zn concentration, indicating that as sediment concentration increased fecundity decreased (Fig. 4.13).

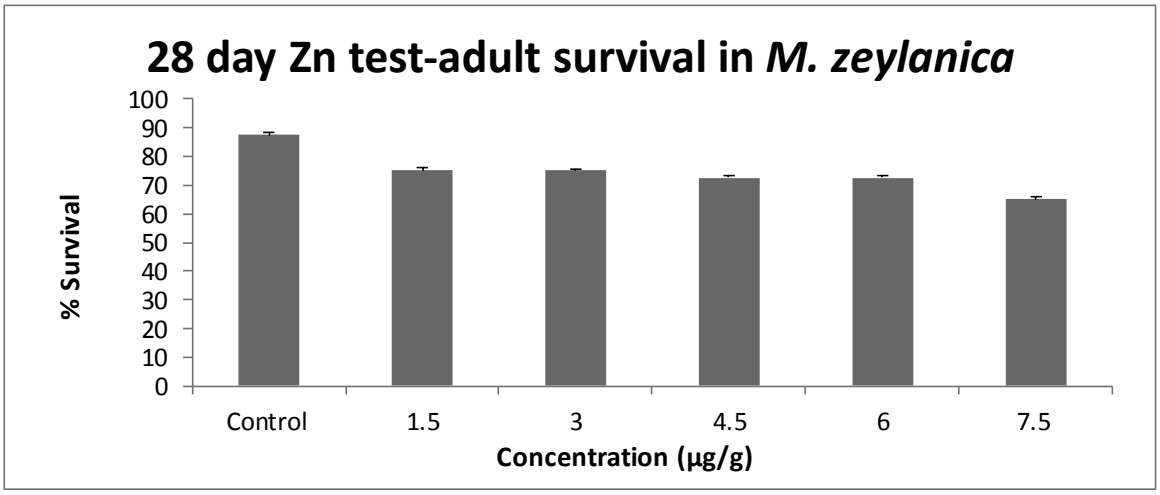

Figure 4. 10 Mean % survival (+1STD) of *M. zeylanica* following a 28 day Zn sediment toxicity test with concentrations ranging from 1.5-7.5 µg/g.

Table 4. 12 ANCOVA: Effect of adult survival on fecundity during 28 day Zn and Cu chronic sediment toxicity test on *M. zeylanica.*

Source	Sum of Squares	df	Mean Square	F	Sig.
Zn adult survival	0.8	1	0,8	0.11	0.92
Zn Fecundity results	9348.9	5	1869.8	25.66	0.001
Cu adult survival	56.1	1	56.1	1.29	0.270
Cu Fecundity result	2265.9	5	453.2	10.49	0.0001

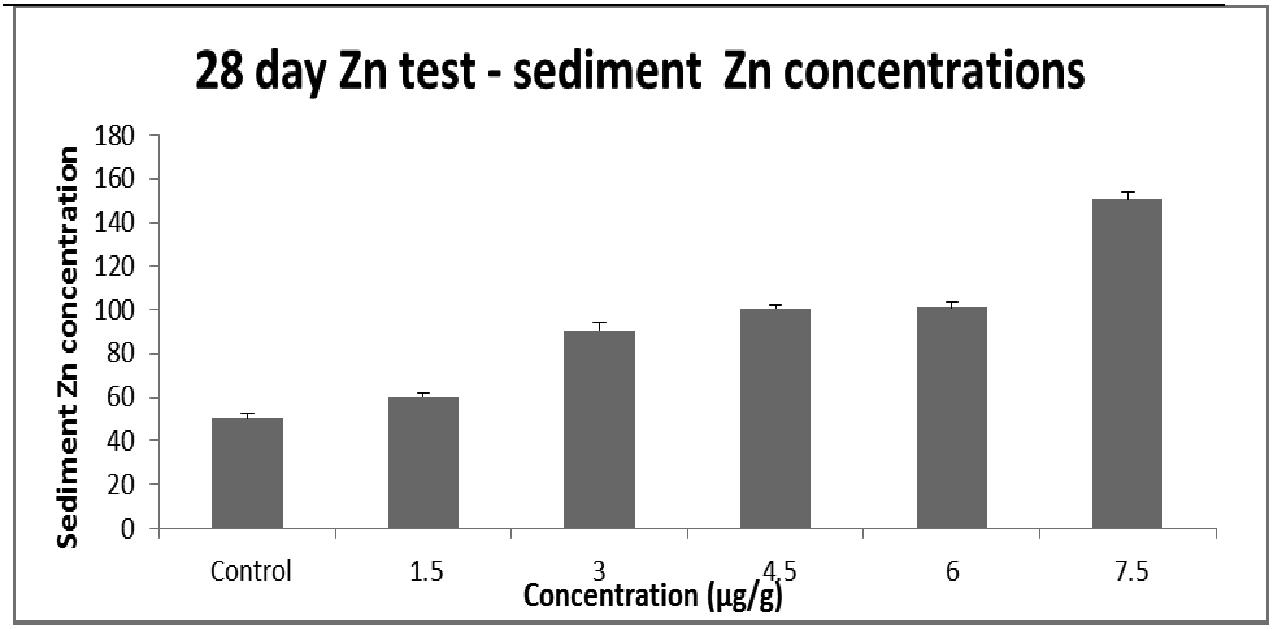

Figure 4. 11 Mean sediment Zn concentration (+1STD) of *M. zeylanica* following a 28 day sediment toxicity test with Zn concentrations ranging from 1.5-7.5 µg/g.

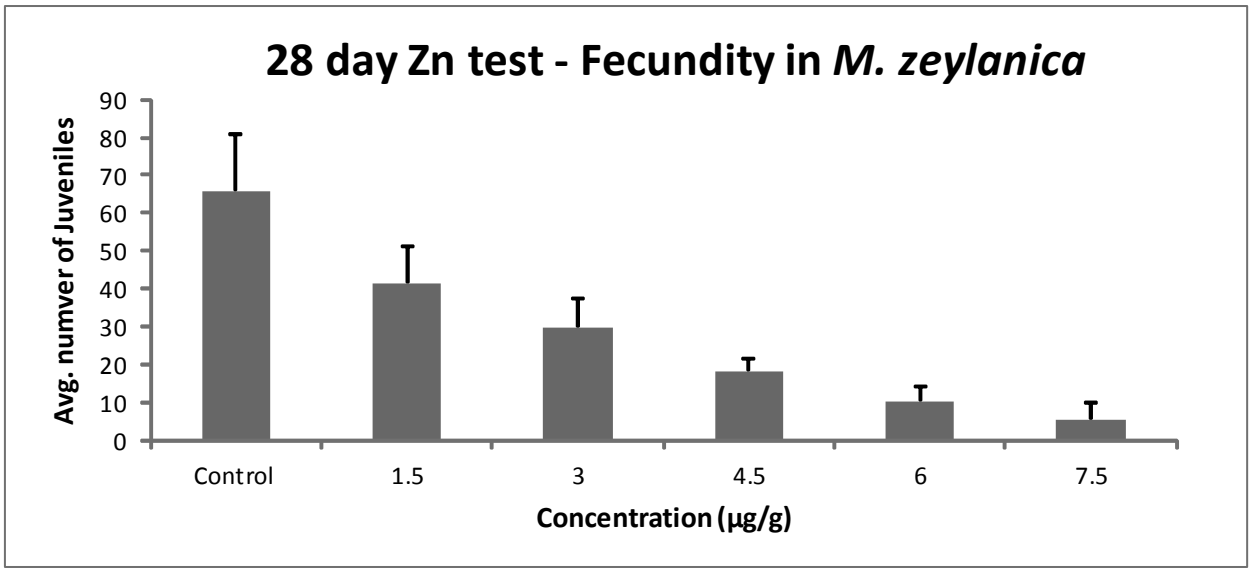

Figure 4. 12 Mean fecundity (+1STD) of *M. zeylanica* in a 28 day Zn sediment toxicity test.

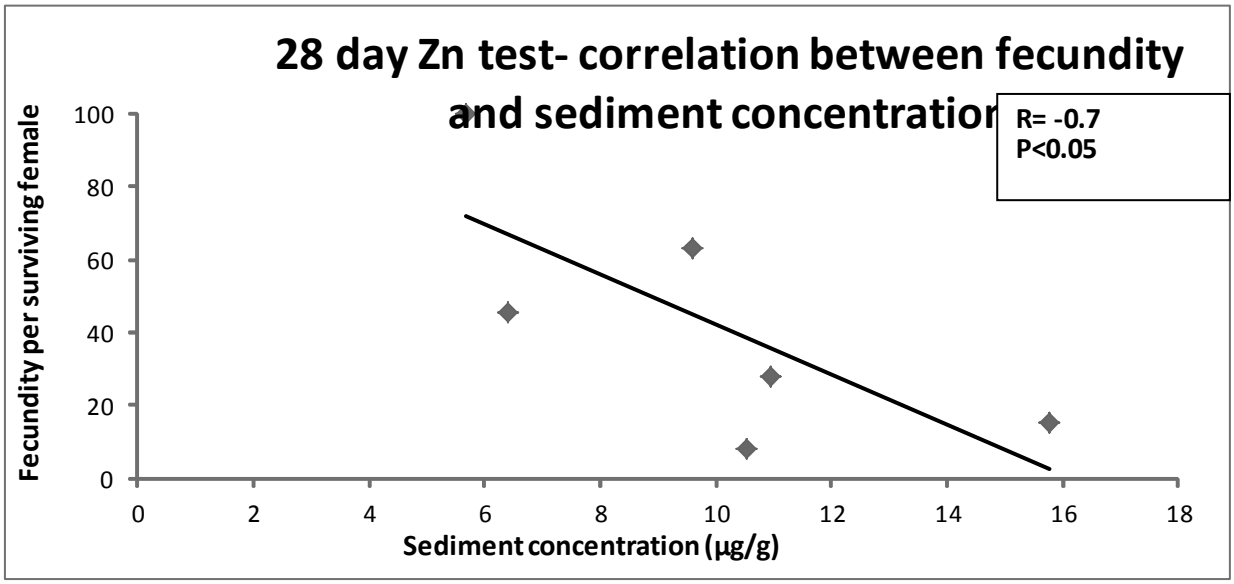

Figure 4. 13 Pearson's correlation between fecundity and sediment Zn concentrations following the 28 day chronic Zn exposure of *M. zeylanica.*

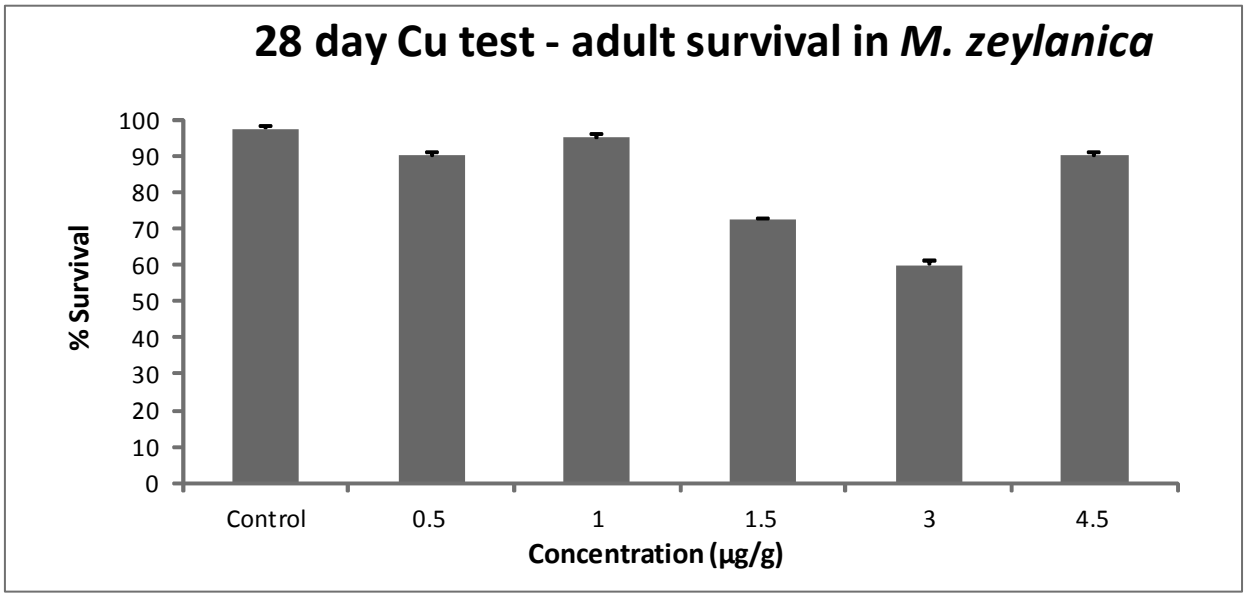

Figure 4. 14 Mean % survival (+1STD) of adult *M. zeylanica* during a 28 day Cu sediment toxicity test with concentrations ranging from 0.5-4-5 µg/g.

4.4.2 28 day Cu Exposure

Adult survival remained high during the 28 day Cu chronic exposure, ranging from 97% in the control to 60% at 3 µg/g. At least 95% adult survival was recorded in four of the six exposure concentrations (Fig. 4.14). EC_{10}, EC_{50} and EC_{90} values for the 28 day Cu concentration were 0.19, 0.84 and 3.85 µg/g, respectively (Table 4.13). Sediment metal concentrations increased progressively with increasing Cu exposure concentration, with the lowest concentration being 23.1 µg/g and the highest 36.6 µg/g (Fig. 4.15) (Table 4.14).

Fecundity gradually decreased with increasing Cu concentration during the 28 day chronic exposure, with the exception of the highest concentration (4.5 µg/g), in which a slightly higher fecundity was recorded than at 3 µg/g (Fig. 4.16). Pearson's correlation test showed a significant negative correlation between exposure Cu concentration and fecundity during the 28 day test (R = -0.65) (Fig. 4.17). The Kruskal Wallis results showed that mean fecundity differed significantly between different Cu concentrations ($p<0.05$) (Table 4.3), while the *post hoc* analysis revealed that significant differences in fecundity were only recorded between the first two exposures (Table 4.4).

Table 4. 13 Probit analysis showing EC_{10}, EC_{50} and EC_{90} of *M. zeylanica* following a 28 day Cu fecundity test.

	EC_{10}	EC_{50}	EC_{90}
Cu 28 day Fecundity	0.19	0.85	3.85

Table 4. 14 Mean sediment concentrations (µg/g) of *M. zeylanica* following a 28 day sediment toxicity test with Cu concentrations ranging from 0.5-4.5 µg/g.

Exposure concentration	Sediment Cu Concentration	STD
Control	23.19	4.92
0.5	26.06	6.68
1.0	26.14	8.14
1.5	26.77	3.90
3.0	36.63	10.07
4.5	38.53	3.63

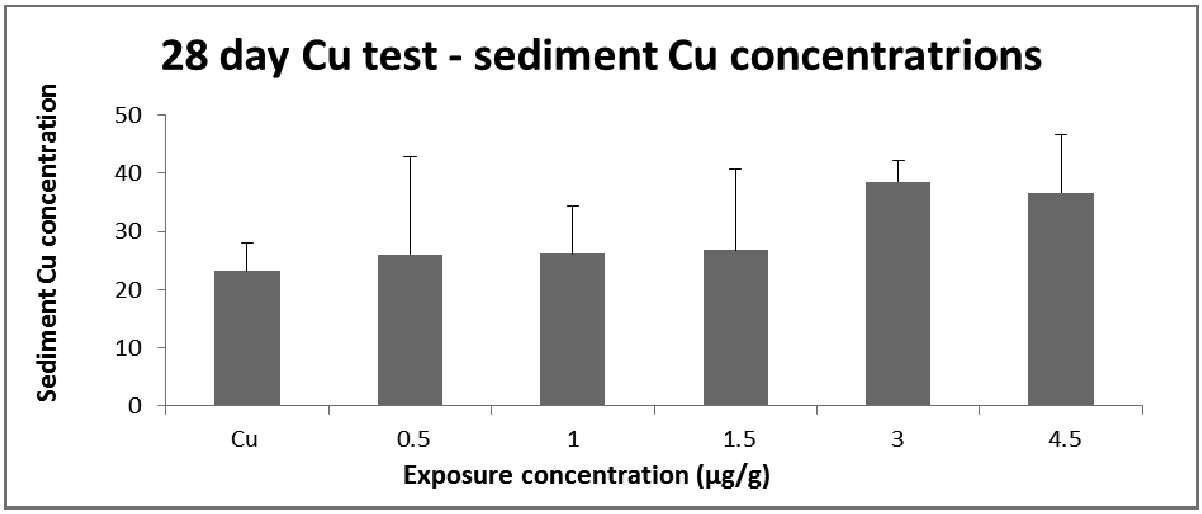

Figure 4. 15 Mean sediment Cu concentrations (+1STD) following a 28 day sediment toxicity test using *M. zeylanica* with Cu concentrations ranging from 0.5.4.5 µg/g.

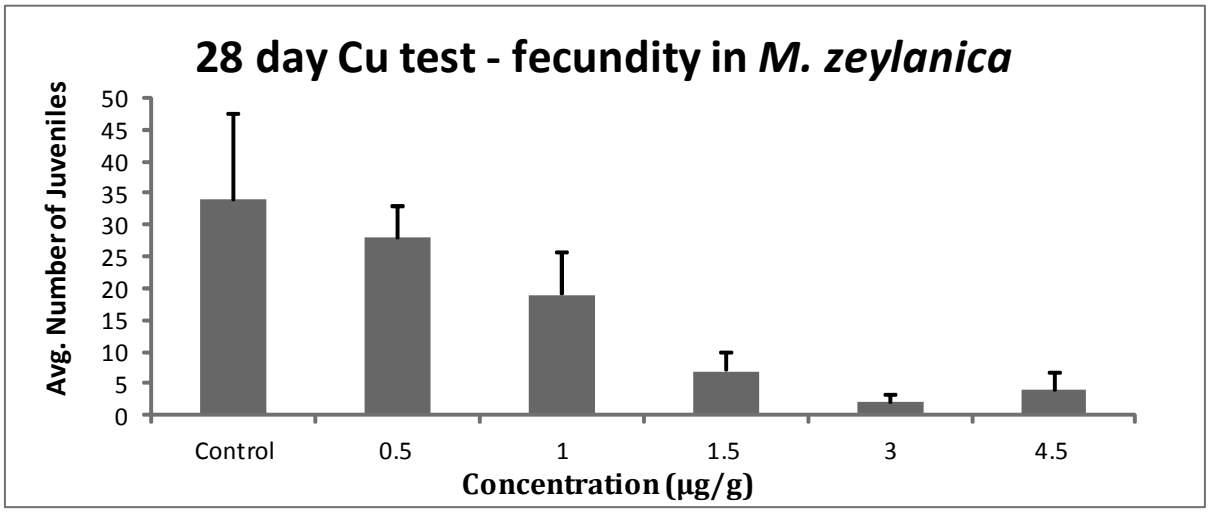

Figure 4. 16 Mean fecundity (+1STD) of *M. zeylanica* following a 28 day sediment Cu toxicity test with concentrations ranging from 0.5-4.5 µg/g.

The mean body length of juvenile amphipods at the end of the 28 day Cu test period decreased with increasing Cu concentration, ranging from 2.1 mm in the control to 1.45 mm in the 4.5 µg/g concentration. The exception was the 3 µg/g exposure, which showed an average length of 2.19 mm, this being considerably higher than the previous concentration (1.5 µg/g), which showed an average juvenile body length of 1.44 mm (Fig. 4.18) (Table 4.15).

The number of size classes decreased with increasing Cu concentrations following the 28 day Cu exposure, with the highest exposure (4.5 µg/g) showing only one class as well as the lowest average length (Table 4.16). Pearson's correlation showed that body length of amphipods correlated negatively with Cu sediment concentration following the 28 day chronic exposure (Fig. 4.19).

Table 4. 15 Mean body length (mm) of juvenile *M. zeylanica* following a 28 day Cu toxicity test.

Cu concentration (µg/g)	Juvenile body length	STD
Control	2.10	1.27
0.5	1.88	0.32
1.0	1.80	0.15
1.5	1.70	0.74
3.0	2.19	0.26
4.5	1.45	0.63

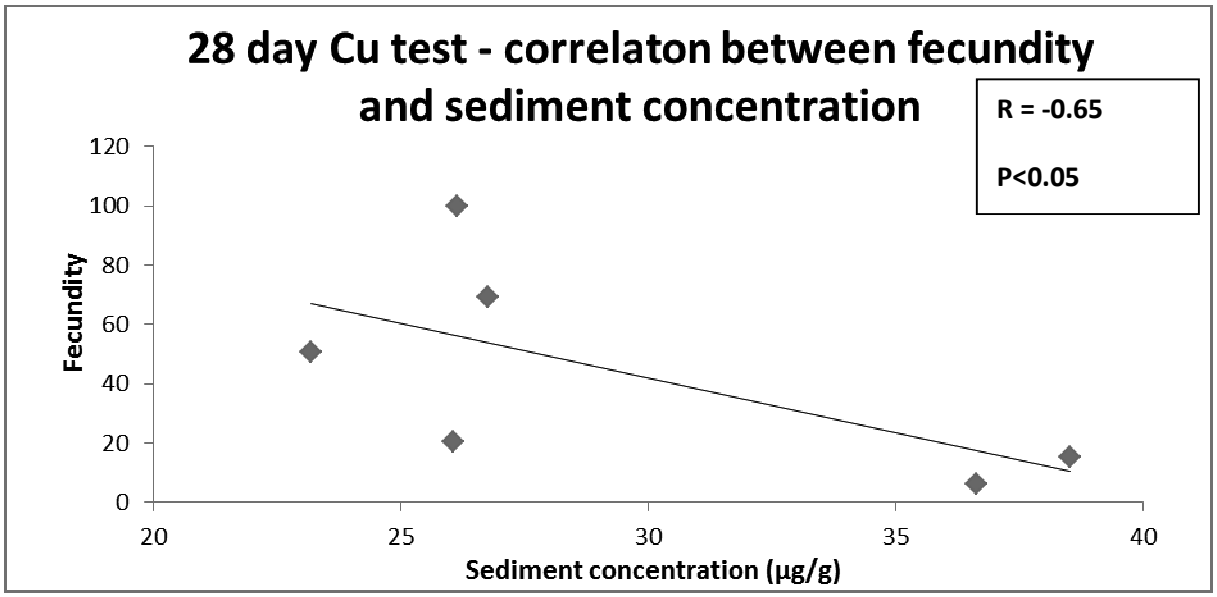

Figure 4. 17 Pearson's Correlation between fecundity and sediment Cu concentrations following the 28 day chronic Cu exposure using *M. zeylanica*.

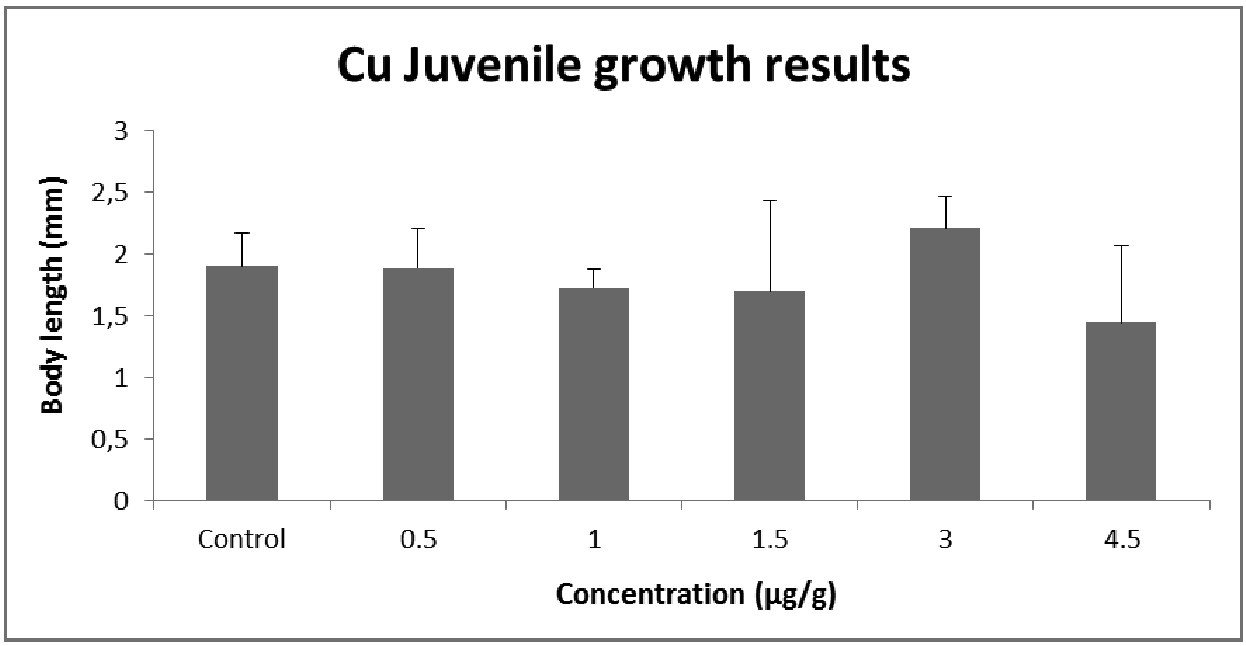

Figure 4. 18 Mean juvenile body length (+1STD) of *M. zeylanica* following a 28 day sediment Cu toxicity test with concentrations ranging from 0.5-4.5 µg/g.

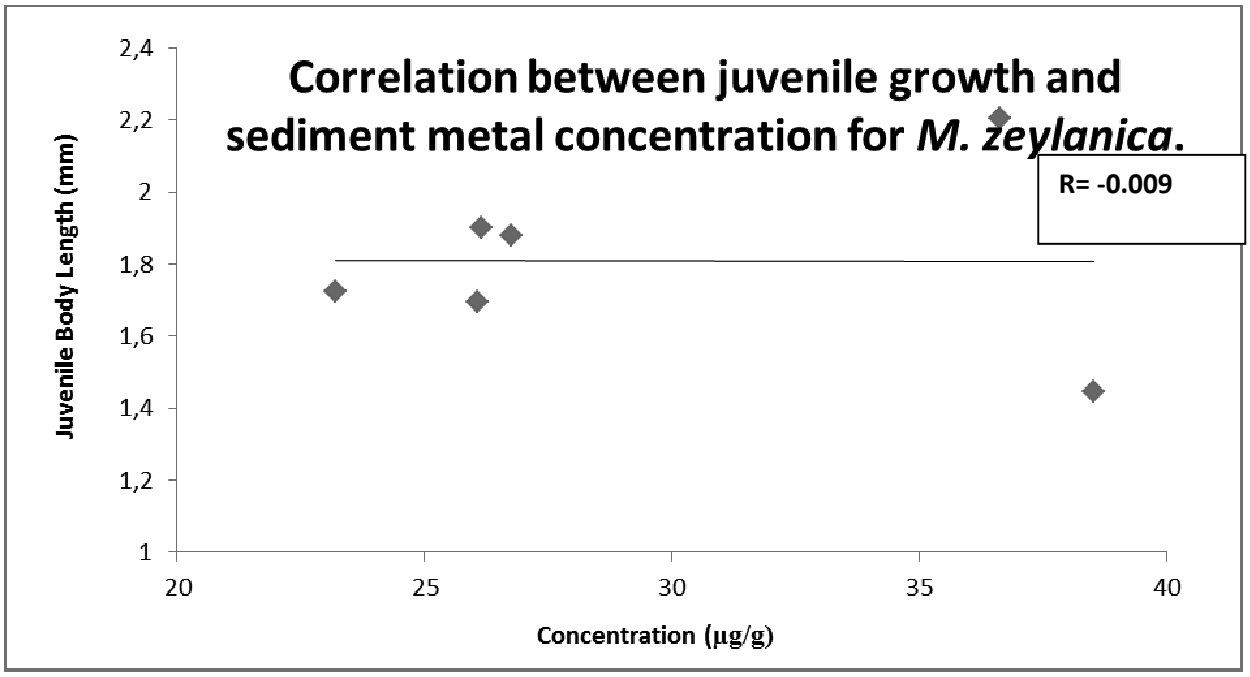

Figure 4. 19 Pearson's Correlation between juvenile length and sediment Cu concentrations following the 28 day chronic Cu exposure using *M. zeylanica.*

Table 4. 16 Size classes of juvenile *M. zeylanica* following a 28 day Cu chronic exposure test.

Cu concentration	Number of juveniles	Number of size classes	Class sizes (mm)	Average length (mm)
Control	17	3	1) 1.00 - 1.90 2) 2.00 - 2.29 3) 2.30 - 2.40	2.10
0.5	17	3	1) 1.00 -1.90 2) 2.00 - 2.29 3) 2.30 -2.40	1.88
1.0	15	2	1) 1.00 -1.90 2) 2.00 - 2.29	1.81
1.5	7	1	1) 1.00 - 1.90	1.69
3.0	1	1	1) 2.00 - 2.290	2.19
4.5	3	1	1) 1.00 - 1.90	1.44

4.5 Field validation experiment – Richards Bay Harbour

4.5.1 Richards Bay Harbour Sediment

Sediment grain size (median phi) ranged between coarse sand at Pelican Island and silt at RBCT and the Bulk (C7) (Fig. 4.20). The percentage mud fraction (silt) similarly ranged between 2-92%, with the highest and lowest mud content recorded at Bulk (C7) and in the Mzingazi Canal, respectively. Organic content (%) were also highest at Bulk (C7) (0.78%), with lowest at Pelican Island (0.01%). Well sorted sediment was found in the Manzimnyama Canal and in the Small Craft Harbour, while poorly sorted sediment occurred in the Mzingazi Canal, Bhizolo Canal and at Bulk (C7)**.**

4.5.2 10-day sediment exposure at six sites in Richards Bay Harbour.

Laboratory methods for soil and Foliar analysis in long-term environmental monitoring prorams methodology was followed for grain-size analysis (Schumacher *et al.* 1995)Pelican Island sediment showed the highest amphipod survival (72.5%), followed by Bhizolo Canal sediment (67%), while Manzimnyama Canal and RBCT showed 60% and 53% survival, respectively. Bulk (C7) and Mzingazi Canal sites showed the lowest survival at 40 and 35%, respectively. The low survival (35%) in Mzingazi Canal sediment was surprising as this area was previously regarded as a relatively uncontaminated region of the harbour. There was no significant difference in amphipod survival between the six sites of exposure in Richards Bay Harbour ($p>0.05$) (Table 4.3).

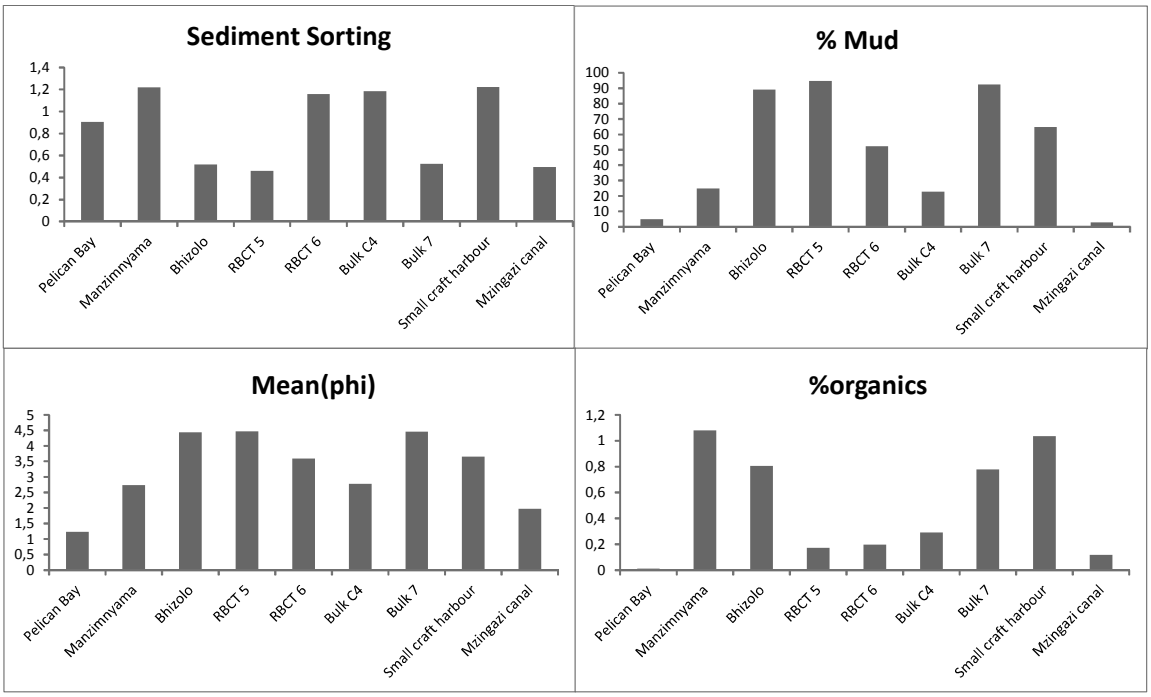

Figure 4. 20 Sediment characteristics (% organics, % mud, median (phi) and sorting coefficient) at nine sites in Richards Bay Harbour.

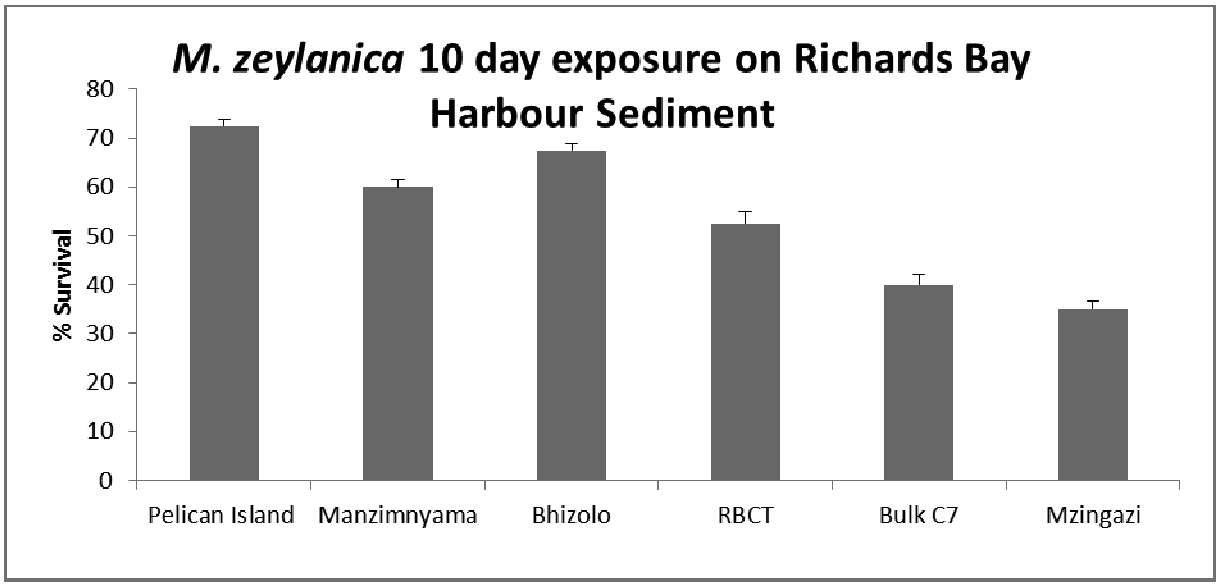

Figure 4. 21 Mean survival (%) (+1STD) of *M. zeylanica* during a 10 day sediment toxicity test on Richards Bay Harbour sediment.

4.5.2.1 Richards Bay Harbour - sediment metal concentrations

Al and Fe: Aluminium and Fe were found to be an order of magnitude higher in concentration than the rest of the metals. This was expected as both Al and Fe are known to be the most common metals in the earth's crust and therefore are naturally high in most areas (Mucha *et al*. 2003).

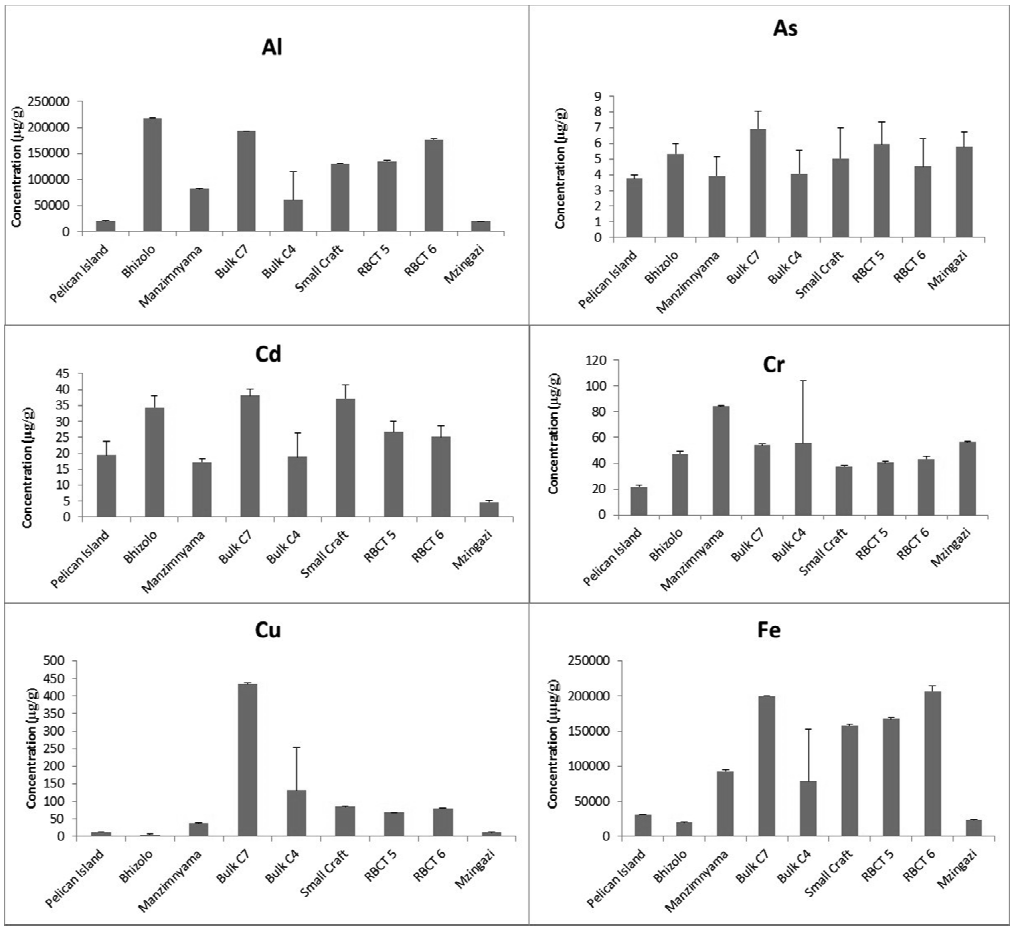

Figure 4. 22 Mean (+1STD) sediment metal concentrations from nine sites in Richards Bay Harbour.

As: Sediment As concentrations were found to be low in Richards Bay Harbour. The highest As concentration was recorded at the Bulk (C7) (6.92 µg/g), while the lowest concentration was at Pelican Island (3.78 µg/g).

Cd: The highest sediment Cd concentration was recorded at the Bulk C7 (37 µg/g), with the lowest sediment recorded in the Mzingazi Canal (4.68 µg/g).

Cr: Sediment Cr concentrations ranged between 21 and 84 µg/g, with the highest Cr recording in the Manzimnyama Canal (84 µg/g) and the lowest concentration recording at Pelican Island (21.37 µg/g).

Cu: Sediment Cu concentrations ranged between 11 and 434 µg/g, with the highest concentration at Bulk (C7) and the lowest concentration in the Mzingazi Canal. The highest Cu concentration was recorded at the Bulk (C7) (434 µg/g), which was considerably higher than the second highest concentration at the Bulk (C4) (131 µg/g). The lowest Cu concentration was recorded in the Mzingazi Canal and Pelican Island (11 µg/g)

Hg: The recorded range of sediment Hg concentration was between 3.5 and 11 µg/g. The highest Hg concentration was recorded in the Mzingazi Canal (11 µg/g), with the lowest recorded at RBCT (5) (3.5 µg/g).

Ni: Sediment Ni concentrations were found to range between 35 and 122 µg/g. The highest Ni concentration was recorded at Bulk (C7) (121.68 µg/g), with the lowest Ni concentration recorded in the Mzingazi Canal (35 µg/g).

Pb: The highest sediment Pd concentration was recorded at RBCT (6) (10 µg/g), while the lowest was recorded in the Mzingazi Canal (5 µg/g).

Zn: Sediment Zn concentrations ranged between 94 and 1487 µg/g, with the highest concentration at the Bulk (C7) and the lowest concentration in the Mzingazi Canal.

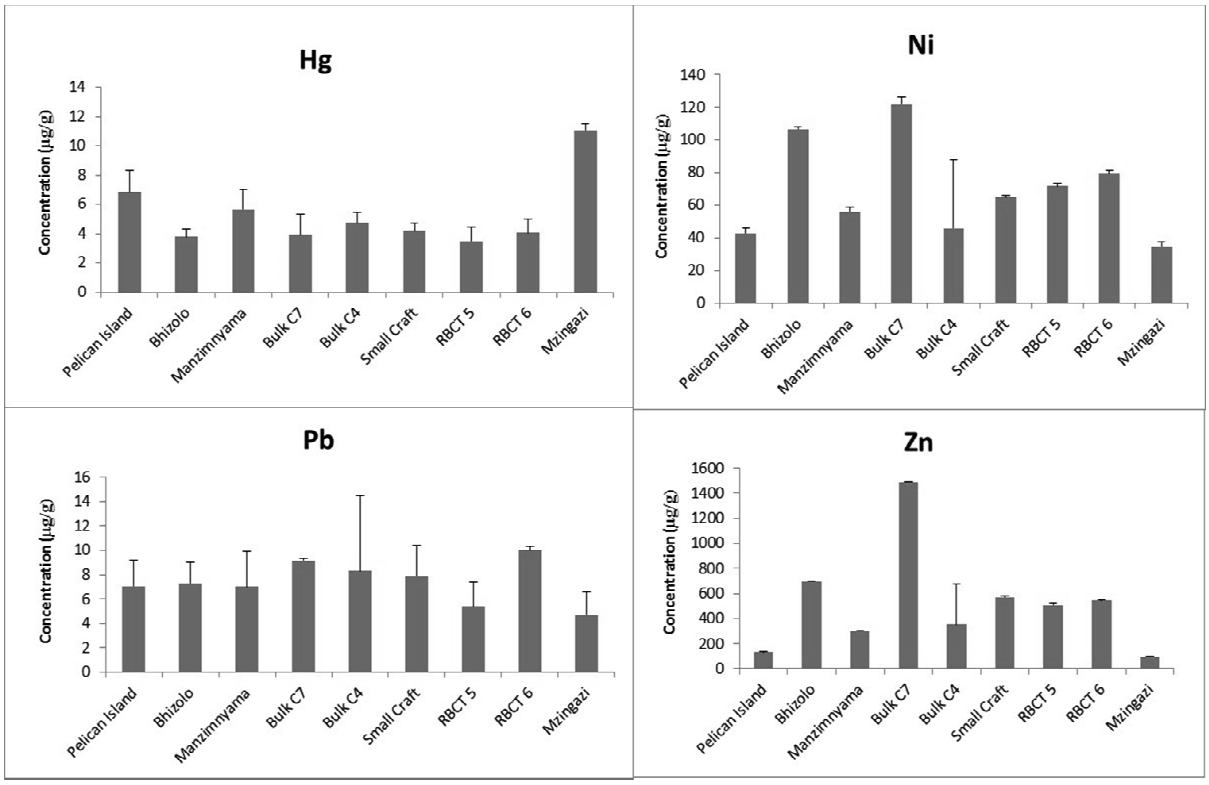

Figure 4. 23 Mean (+1STD) sediment metal concentrations from nine sites in Richards Bay Harbour.

4.5.2.2 Richards Bay Harbour - metal accumulation in tissue

Even though metal accumulation was evident for all metals, tissue concentrations in *M. zeylanica* remained below sediment concentrations for most metals. Arsenic, Hg and Cr showed considerable accumulation in *M. zeylanica* as compared to the rest of the metals following the 10 day field validation exposure using sediment collected from six sites within Richards Bay Harbour (Fig. 4.6, Fig. 4.27).

Tissue metal concentrations showed positive correlations with sediment metal concentrations, meaning that tissue concentrations increased with sediment metal concentrations (Fig. 4.28, Fig. 4.29). Significant positive correlations were observed in Ni and Zn. Copper showed the highest correlation (R = 0.81), while Al and Fe showed the lowest correlation (R = 0.26, R = 0.23), respectively.

As: The highest As tissue concentration was recorded at RBCT (6) (5 µg/g), with the lowest concentration recorded at Pelican Island (3.5 µg/g).

Cd: The highest Cd tissue concentration was recorded in the Bhizolo Canal (27 µg/g), with the lowest record being the RBCT (6) site (10.2 µg/g).

Cr: The highest Cr tissue concentration was recorded in the Manzimnyama Canal (89 µg/g), with the lowest record concentration at Pelican Island. (40 µg/g)

Cu: The highest Cu tissue concentration was recorded at the Bulk (C7) (59 µg/g), while the lowest was recorded at Pelican Island (11 µg/g).

Hg: The highest Hg tissue concentration recorded was at RBCT (6) and in the Mzingazi Canal (8 µg/g), with the lowest concentration being recorded in the Manzimnyama Canal (6 µg/g).

Ni: The highest Ni tissue concentration was recorded at the Bulk (C7) (36 µ g/g), with the lowest concentration in the Mzingazi Canal (24 µg/g).

Pb: The highest Pb tissue concentration was recorded in Pelican Island (7 µg/g), with the lowest in the Mzingazi Canal (5.1 µg/g).

Zn: Zinc tissue concentrations ranged between 89 and 228 µg/g. The highest tissue concentration was recorded at the Bulk (C7) (228 µg/g), with the lowest being Pelican Island (89 µg/g).

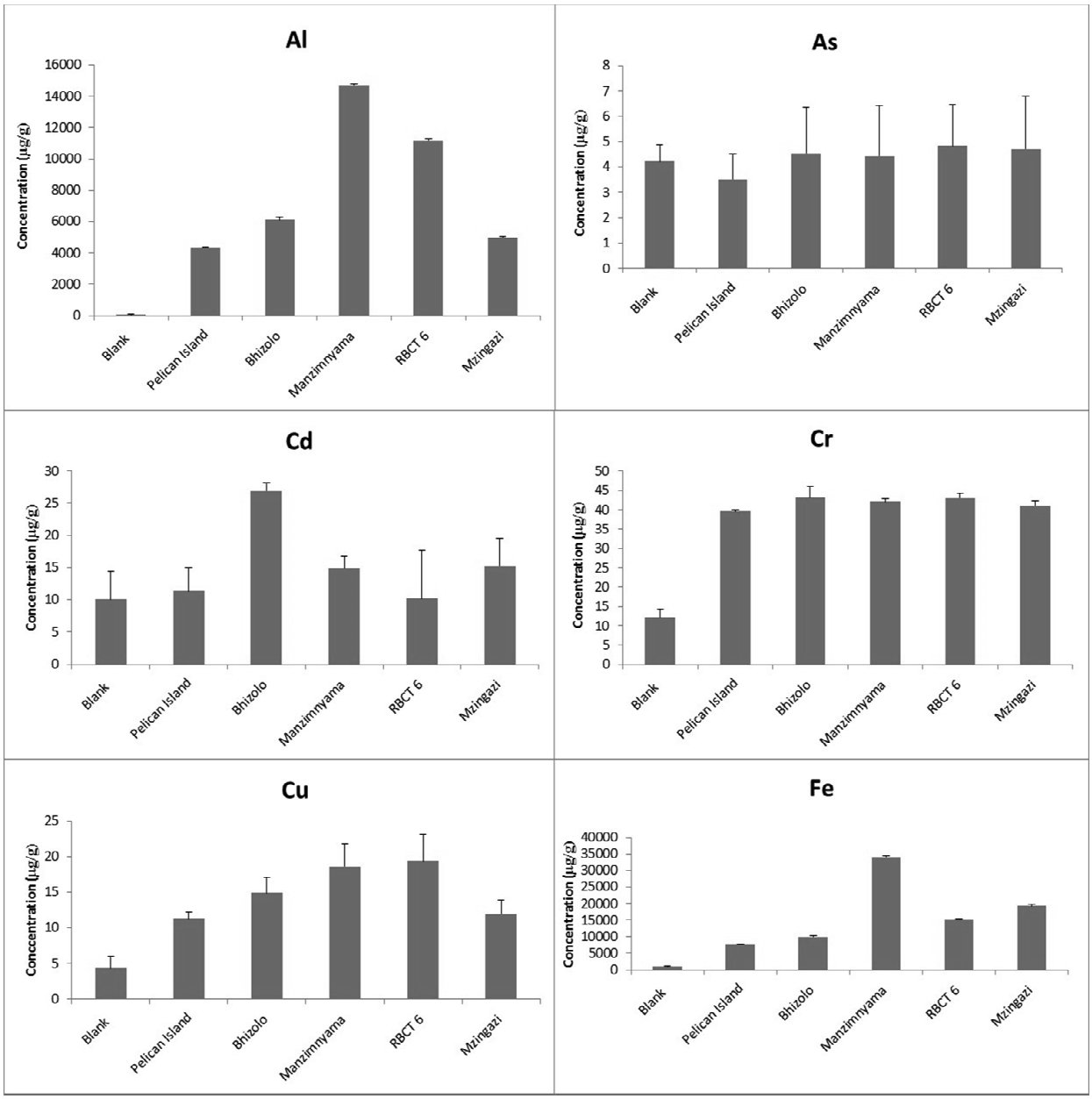

Figure 4. 24 Mean (+1STD) tissue metal concentrations in *M. zeylanica* following a 10 day field validation exposure on Richards Bay Harbour sediment.

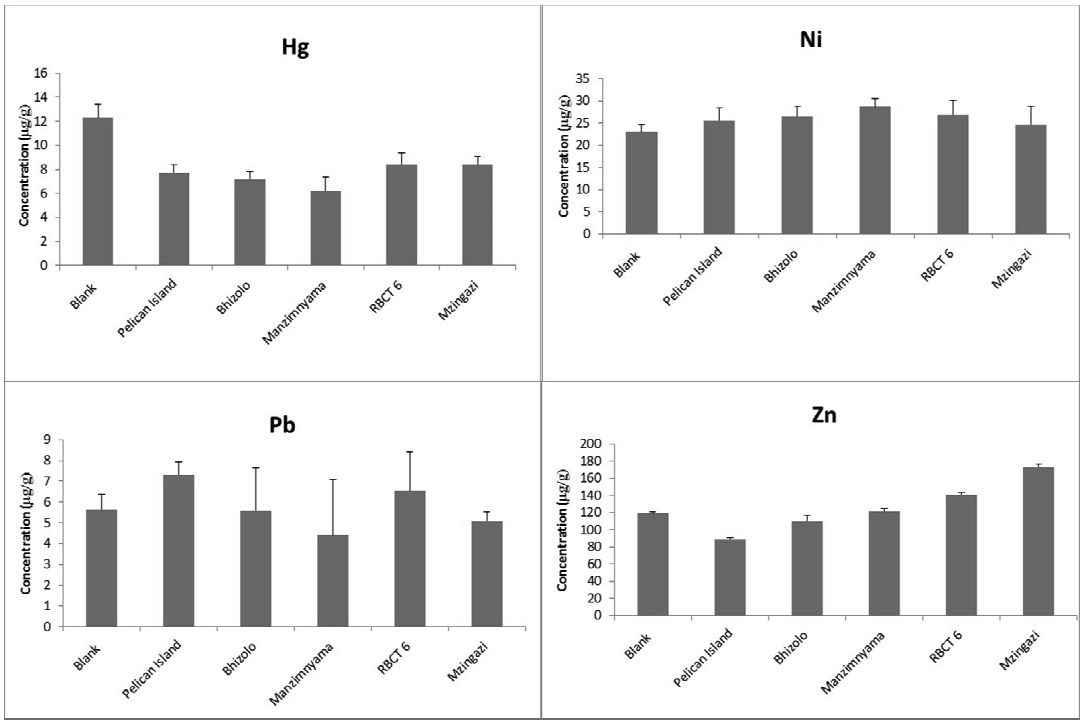

Figure 4. 25 Mean (+1STD) tissue metal concentrations in *M. zeylanica* following a 10 day field validation exposure on Richards Bay Harbour sediment.

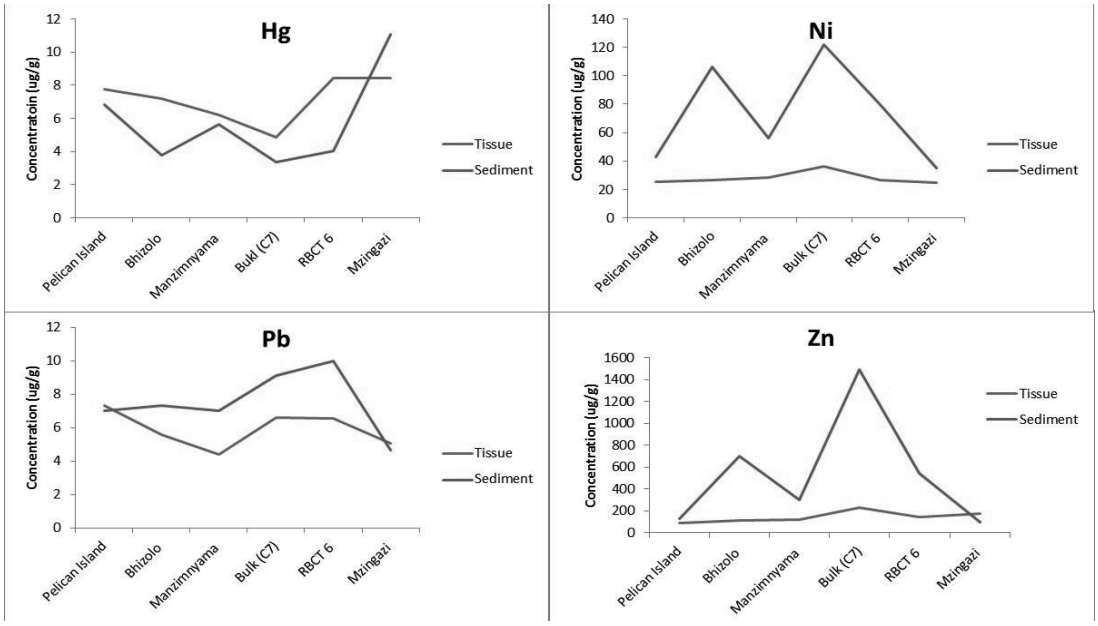

Figure 4. 26 Richards Bay Habour sediment metal concentrations and *M. zeylanica* tissue metal concentrations following a 10 day toxicity exposure.

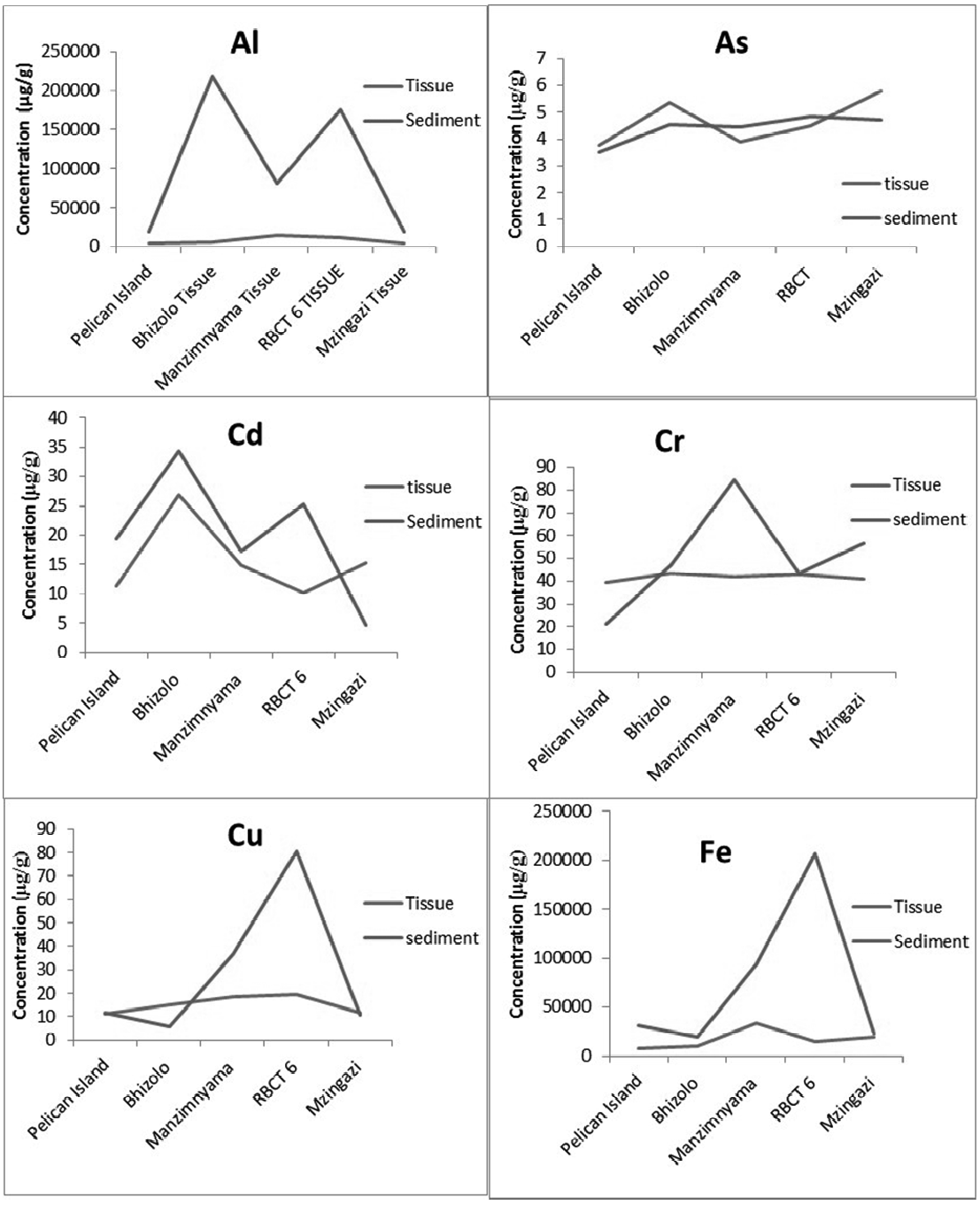

Figure 4. 27 Richards Bay Harbour sediment metal concentrations and *M. zeylanica* tissue metal concentrations following a 10 day toxicity exposure.

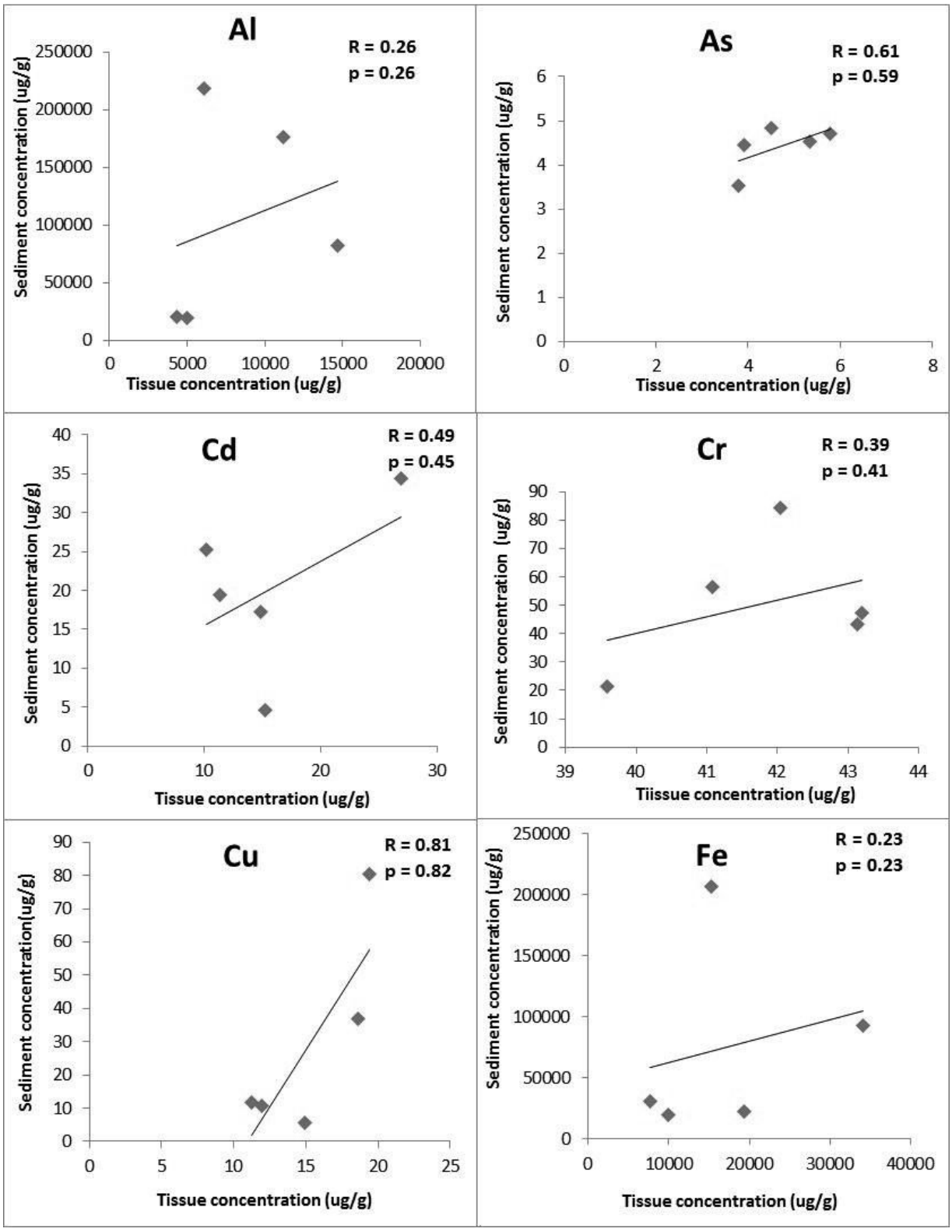

Figure 4. 28 Pearson's Correlation between sediment and tissue metal concentrations following a 10 day validation exposure on Richards Bay Harbour sediment.

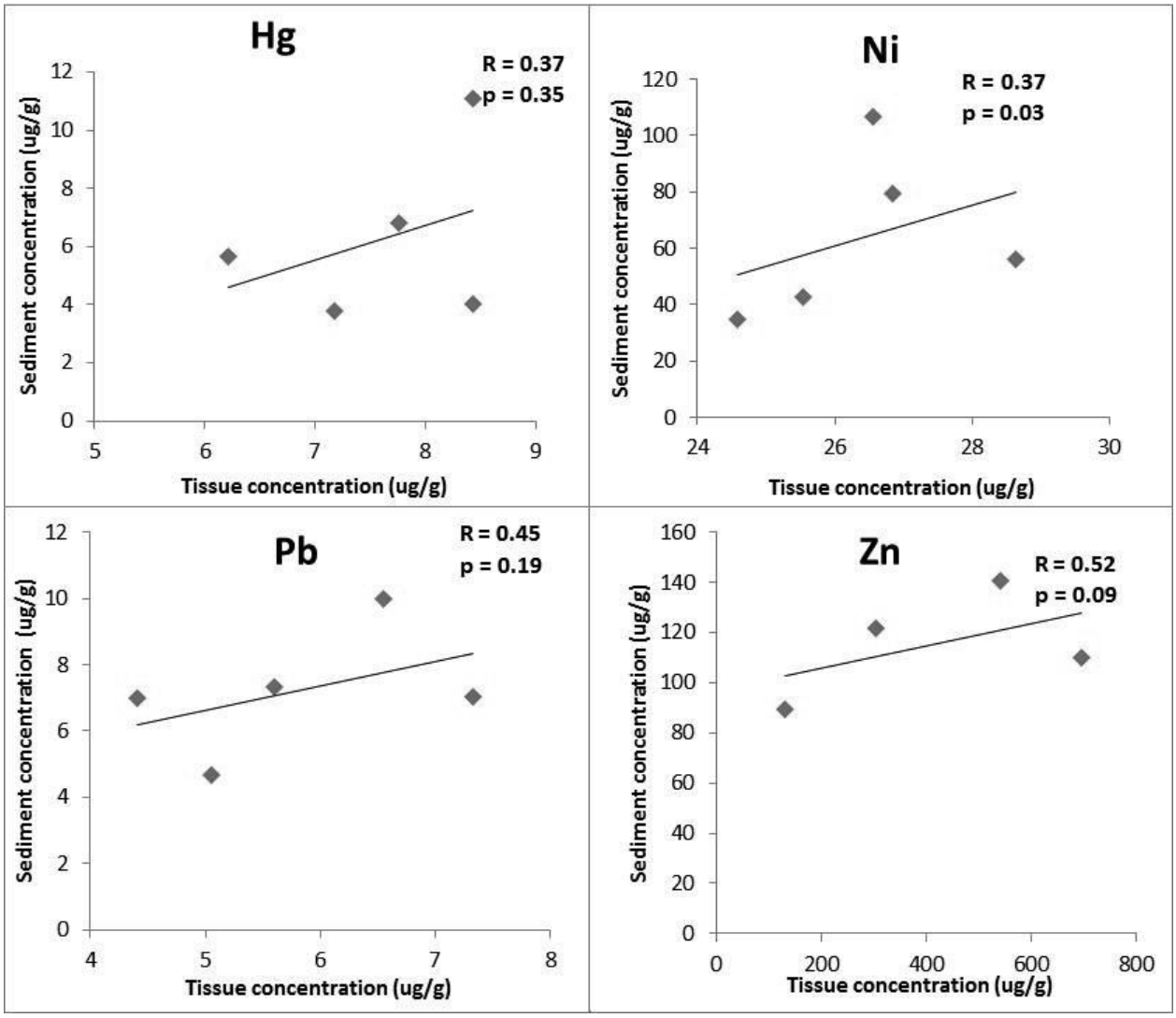

Figure 4. 29 Pearson's Correlation between sediment and tissue metal concentrations following a 10 day validation exposure on Richards Bay Harbour sediment.

CHAPTER 5

DISCUSSION

5.1 *Melita zeylanica* Bioassays

Melita zeylanica is a gammarid species, and according to Thomas (1993), gammarid amphipods are suitable for toxicity studies as they are of high ecological importance and usually occur in high densities. Furthermore, gammarid species generally demonstrate a degree of niche specifity and are largely reported in the literature as sensitive to most pollutants and toxicants (Reish 1993). Their low dispersal capabilities and their tendency to respond immediately to pollutants that may threaten their health and population, causes gammarids to be among the favourite organisms used for toxicity testing worldwide (Boets *et al.* 2012). Comparisons of sensitivity of *Melita spp*. with other amphipods indicated that *Melita spp*. are among the most sensitive to metals (Gale *et al*. 2006; King *et al*. 2006a). The *Melita spp*. have been used in toxicity testing, *M. plumulosa* (King *et al.* 2006a) for instance, has been used in Australia as a biomonitor species and *M. awa, M. petronioi, M. palmata*, as well as *M. matilda (*EPA database 2013*),* have all been identified and investigated quite extensively in toxicity testing worldwide.

Amphipod bioassays are affected by physico-chemical parameters such as temperature and salinity, which makes it difficult for inter-laboratory comparison (Marsden 2002). It would, therefore, be ideal to carry out experiments simultaneously under similar physico-chemical parameters in order to adequately compare sensitivity of different species. This, however, is difficult as bioassays in different labs are conducted independently of each other, thus are unlikely to have exactly the same environmental characteristics.

5.1.1 96hr Cd toxicity test

To ensure some form of standardisation, 96hr Cd water only, reference toxicity tests are used to compare amphipod sensitivity to trace metals and there is extensive literature on the relative sensitivity of amphipods to trace metals (ASTM 2008). In this study, a 96hr Cd water-only reference toxicity test was conducted and results showed a LC_{50} value of 1.176 mg/l (Table 4.2) for *M. zeylanica* when exposed to Cd concentrations ranging from 0.5 to 8 mg/l (Fig. 4.1). This finding was comparable to that of other amphipods used in toxicity testing, such as *Grandidierella lignorum*, *Corophium triaenonyx*, *G. japonica* and *Gammarus locusta,* which were found to have Cd LC_{50} values of 1.1 mg/l, 1.6 mg/l, 1.2 mg/l and 0.9 mg/l, respectively (ASTM 2008, Vivier 2010). When compared to some reports on a number of other species, *M. zeylanica* was found to be considerably more sensitive to Cd, for instance Onorati *et al.* (1999) reported LC_{50} values of 2.9 mg/l and 4.3 mg/l for *Corophioum orientale* from two uncontaminated systems in France, while Ciarelli (1994) reported a LC_{50} value of 5.3 mg/l for *C. volutator* in the Netherlands. Lee *et al.* (2005) also reported a staggering Cd LC_{50} of 9.3mg/l for *Eohaustorius estuarius.* The absence of sediment did not seem to adversely disturb behaviour and reaction of amphipods to Cd as evidenced by the high survival of amphipods in the control exposure (Fig. 4.1). The *post-hoc*

analyses (Table 4.4) revealed that significant differences in survival were only between the first three (lower concentration) exposures, suggesting a rapid drop in survival at relatively low Cd concentrations. These results indicate that *M. zeylanica* is sensitive to Cd which suggests a general sensitivity to metals, and thus *M. zeylanica* could potentially be a good biomarker species for toxicity testing.

5.1.2 96hr Ammonia test

Ammonia toxicity has been found to be an important confounding variable in toxicity testing (Maranho *et al.* 2010). Benthic organisms, in particular, are in close contact with the sediment which makes them very susceptible to ammonia as sediments provide a substrate for bacteria and other biota that excrete ammonia, as compared to the water medium. Ammonia, notably un-ionidized ammonia, has been shown to be acutely toxic to both freshwater and marine amphipods (Khon *et al*. 1994; Prenter *et al.* 2004). During this study, *M. zeylanica* was found to be very sensitive to ammonia, with a LC_{50} value of 17.0 mg/l. Khon *et al.* (1994) listed LC_{50} values for four estuarine amphipods*; Rhepoxynious abronius* (84.5 mg/l), *Eohaustorius estuarius* (168.4 mg/l), *Ampelisca abdita* (54.4 mg/l) and G*randidierella japonica* (203.8 mg/l). Based on these results, three of the amphipods were regarded as relatively sensitive to ammonia, with *G. japonica* being less sensitive. Van den Heuvel-Greve *et al*. (2007) reported a lower ammonia LC_{50} value of 35 mg/l for *Corophium volutator* during a 10 day exposure, than previously reported for this species. These authors emphasised that the salinity under which the test is conducted plays a crucial role in the sensitivity of organisms and that ammonia toxicity increases at lower salinity. In this study, a salinity of 25 was maintained, which could have contributed to the lower tolerance of *M. zeylanica* to ammonia (17.03 mg/l) (Table 4.5) as compared to other studies which conducted bioassays at salinities of ±30. *Melita zeylanica* showed a high survival rate in the first three exposures (1, 5 and 10 mg/l), with a 100% mortality in concentrations above 10 mg/l (20 and 50 mg/l) (Fig. 4.2). This result indicates an abrupt drop in ammonia tolerance between 10 and 20 mg/l concentrations, which suggests that ammonia intolerance occurs rather rapidly in *M. zeylanica*. A 96hr test was conducted for ammonia sensitivity in this study, which is a relatively short duration and results of the test may not necessarily reflect the long term sensitivity of *M. zeylanica* to ammonia. A longer duration bioassay would, therefore, reflect a more realistic ammonia sensitivity of *M. zeylanica*. The relatively high intolerance of *M. zeylanica* to ammonia would need to be considered in future toxicity studies and care taken not to exceed its tolerance limits. In this study, the LC_{50} value of total (ionidized and un-ionidized) ammonia toxicity for *M. zeylanica* was reported, some studies, however, mainly report on ionidized ammonia (NH_4^+).

5.1.3 96hr Salinity test

Estuaries are often referred to as hostile environments, mainly due to rapid salinity fluctuations (Owen and Forbes 2002). Delgado *et al*. (2011) reported that high salinity affected osmoregulation in amphipods, thus affecting their population dynamics. Different species have different tolerance to salinity changes, an ideal organism for toxicity testing, therefore, has to have a wide salinity tolerance, so it can be used in bioassays at various salinities (Kotta *et al*. 2013).

In this study, a water-only 96 hr salinity tolerance test was conducted under a relatively wide range of salinities (0-40) (Fig. 4.3). The results showed that *M. zeylanica* is a euryhaline species that can tolerate and survive in a wide range of salinities, survival was consistently high in all exposures, except in freshwater. A 98% survival was recorded at a salinity of 40, the highest salinity of the experiment (Fig. 4.3). The LC_{50} for salinity was not determined as survival of amphipods did not present a linear progression, i.e. *M. zeylanica* was highly tolerant to high and low salinities, and showed 100% mortality at 0, indicating that *M. zeylanica* is tolerant of low salinities, but intolerant of freshwater. This finding was in agreement with other studies such as Masikane *et al*. (2014), who reported that *G. lignorum* tolerated salinities within the range of 7-42 µg/g indicating, that *G. lignorum* is highly tolerant to salinity and that salinity was less likely to affect the outcome of the bioassay. Lee *et al.* (2005) also reported similar findings with *Gammarus aequicauda,* which showed survival and growth in a relatively wide range of salinities 2-40. Teske and Wooldridge (2004) reported that true estuarine species tend to be independent of salinity and that for most South African estuarine species, salinities between 5 and 55 are relatively non-lethal, Teske and Wooldrige (2003) also reported that estuarine amphipods tend to be tolerant to high salinity fluctuations, however, abundance is affected by temporal extent of exposure, i.e. only if elevated salinity persist will the organism be affected. It can be concluded, therefore, that salinity is not a confounding factor for *M. zeylanica* bioassays.

5.1.4 10 Day Zn and Cu Bioassays

Amphipod survival decreased progressively with increasing Zn concentration. A 100% survival was recorded in the control exposure, indicating that test conditions were suitable to conduct the experiment. 70% of the amphipods survived in the lowest Zn concentration, while 30% survival was recorded in the highest exposure (Fig. 4.4). A 100% mortality was, therefore, not realised with the 10 day Zn bioassay, indicating that *M. zeylanica* could have survived at even higher concentrations. The LC_{50} for Zn, however, was found to be rather low (9.15 µg/g) (Table 4.6) as compared to findings from other studies, suggesting that *M. zeylanica* is very sensitive to Zn contamination. Vivier (2010) for instance, reported LC_{50} values of 25.8 µg/g for *G lignorum* and 20.1 µg/g for *C. triaenonyx,* while Bat and Raffaeli (1998) reported Zn LC_{50} values of 14.2 µg/g for *C. volutator.*

An 85% survival was recorded following the 10 day Cu bioassay control exposure, which indicated that experimental conditions were acceptable to conduct the experiment (Fig. 4.7). The lowest (2 µg/g) and highest (32 µg/g) Cu concentrations showed a 75% and 30% survival in *M. zeylanica*, respectively, with a LC_{50} value of 11.76 µg/g (Table 4.8). Marsden and Wong (2001) reported a LC_{50} value of 55 µg/g for the New Zealand amphipod, *Paracorophium excavatum*. *Melita zeylanica* Cu LC_{50} value did, however, compare favourably with that of *Grandidierella locusta*, in which a LC_{50} value of 8 µg/g Cu was reported (Costa *et al.* 1998). These results may suggest that Cu is highly toxic to *M. zeylanica* and that survival is affected even at low concentrations. When compared to other studies, *M. zeylanica* was found to be relatively sensitive to Cu and Zn.

Considering LC_{50} values, Zn and Cu were found in this study to be almost equally toxic to *M. zeylanica*, i.e. LC_{50} 11.76 µg/g and 9.15 µ/g for Cu and Zn (Table 4.8, Table 4.6), respectively, suggesting that *M. zeylanica* is equally sensitive to both Zn and Cu. This finding was not entirely in agreement with the general understanding that Cu is more toxic than Zn (Abel 1989, ASTM 2008). The LC_{90}, however, was found to be lower in Cu than in Zn i.e. LC_{90} values of 78.70 µg/g and 233.51 µg/g for Cu and Zn, respectively (Table 4.8 and 4.6), suggesting that *M. zeylanica* is less sensitive to Zn at higher concentrations as compared to Cu.

5.2 Bioaccumulation and Bioconcentration.

Rainbow and White (1989) emphasized that the ability to accumulate trace metals is one of the main pre-requisites for a sediment bioassay test organism. According to Rainbow (2007), organisms handle metal contamination differently, basically, physiological contamination occurs as a contaminant or metal binds to an enzyme thus preventing it from functioning properly. Two main processes used by organisms to control metal contamination are detoxification and excretion (Rainbow 2002). Detoxification refers to the process of metabolisation of metal before it can bind to an enzyme thus preventing its toxicity and the contaminant can be stored in the organism's body fat, while excretion refers to metal elimination from the organism's body before it can cause the organism harm (Marsden and Rainbow 2004). Should the rate of uptake exceed that of excretion and detoxification, then the chances of metabolically active metal reaching toxic levels and eventually exceeding the lethal threshold are increased (Marsden and Rainbow 2004, Simpson and Batley 2007; Pastorinho *et al.* 2009). According to Rainbow (2002), most decapod crustaceans have evolved strategies to regulate metal concentrations, while some crustaceans such as barnacles and amphipods tend to accumulate metals in their tissues. Furthermore, metal absorption or metabolisation in organisms depends heavily on both the concentration and duration of exposure to the organism (Marsden and Rainbow 2004).

Melita zeylanica displayed body accumulation of both Zn and Cu with increasing sediment concentration following the 10 day exposures. It was, however, noticed that Zn was accumulated to

much higher concentrations, i.e. the range of body tissue accumulation was much higher for Zn (46.7-252.0 µg/g) than Cu (51.6-100.5 µg/g) (Table 4.7, Table 4.9). These results were found to compare favourably with other studies elsewhere. Marsden and Wong (2011) reported Cu concentration ranges between 90-207 µg/g and 84-550 µg/g in two amphipods, *P. excavatum* and *C. volutator,* respectively, while body Zn concentrations ranged between 79-330 µg/g for *C. volutator* (Bat and Raffaeli 1998).

King *et al.* (2006a) reported that *Melita* amphipods are considered one of the most sensitive amphipod genera to metals, such that *M. awa* has been successively used for routine whole sediment toxicity testing in New Zealand. According to King *et al.* (2006a), *M. awa* is one of the most sensitive amphipod species tested to date and the Cu accumulation range in this species was found to be 110-330 µg/g, while the Zn range was 110-370 µg/g following a 10 day acute toxicity study. Another species belonging to the Genus *Melita*, *M. plumulosa*, has also been reported by King *et al*. (2006a) to be a potential benchmark species for whole sediment acute and chronic toxicity tests. This was due to its relative sensitivity to trace metals, with body tissue concentrations reaching a maximum of 250 µg/g and 650 µg/g for Cu and Zn, respectively. In this study, however, initial metal background concentrations were not measured. It is, therefore, difficult to determine the actual factor by which the metal concentration increased from the initial background following the 10 day acute exposure. King *et al.* (2006a) did mention that initial Cu and Zn background concentrations were high prior to testing and, therefore, only increased by a factor of three or five times their initial background.

Geographical heterogeneity of a test organism is highly significant in bioassays as endemic organisms reflect environmental conditions more accurately (Anderson *et al*. 2008). It is, therefore, important that a South African benchmark species be identified and that potential biomarker species be studied and become well understood to enable their use in toxicity studies. Apart from a recent study by Vivier (2010) on the nearby Nhlabane Estuary, there is no historical data on amphipod metal bioaccumulation for any KwaZulu-Natal estuaries.

5.3 Chronic toxicity

Chronic exposure of *M. zeylanica* to Zn and Cu showed high adult survival throughout (Fig. 4.10, Fig. 4.14), suggesting that the chosen concentrations were suitable for the chronic fecundity and growth experiments. The co-variance test revealed that adult mortality during the toxicity tests did not significantly affect fecundity in both Zn and Cu chronic bioassays, suggesting that changes in fecundity were due to metal concentrations.

Growth and fecundity end-points are commonly used in toxicity studies to determine the impact of contaminants on population density. Marsden *et al.* (2000) reported that growth and reproduction in amphipods were affected by high Cu levels as well as sediment characteristics, while Conradi and Depledge (1998) reported that both Cu and Zn reduced amphipod growth as well as fecundity. In the present study, juvenile body length was negatively correlated with Cu while fecundity was negatively correlated with both Zn and Cu (Fig. 4.19, Fig. 4.13, Fig. 4.17). These results were in agreement with the literature (McGee *et al.* 1993; Redmond *et al.* 1994). In contrast, Surtikanti and Heyne (2000) reported average juvenile growth between 2.7 and 3 mm following a 14 day exposure of five *Corophium spp*. and attributed the difference in growth to feeding rate as well as organic content of sediment. According to Phillips *et al.* (1994), organic carbon and substrate are crucial factors determining the health of benthic species. This, once again, highlights the difficulty in comparing inter-laboratory studies as conditions under which experiments were conducted play an essential role. Marsden *et al.* (2000) reported juvenile growth ranging between 1.5 and 1.8 mm for the estuarine amphipod, *Paracorophium excavatum*. These authors emphasised that even though growth was negatively affected by increasing Cu concentrations, organic content as well as feeding rate also influenced growth rate of *P. excavatum*.

According to Manyin and Rowe (2006), chemical exposure may result in increased metabolic costs of organisms by triggering energetically expensive defence mechanisms, such as excretion, neutralization and metabolism of toxins together with repair and replacement of damaged tissue. Werner and Nagel (1997) also stated that there may be synthesis of proteins responsible solely for repairing and replacement of tissue and that this process requires an extensive amount of energy. This suggests that there was an apparent reduction in energy available for reproduction in amphipods exposed to increasing concentrations of Cu and Zn and that increased energy was allocated to maintenance and survival of the amphipods, instead. McGee *et al*. (1998) also emphasised that juveniles are more sensitive to metal toxicity due to their relatively large surface area, as small amphipods accumulate metals faster than adults, which will affect their growth and survival. The high survival rate of juveniles in this study, indicates that exposure concentrations were low enough to affect their growth without killing them.

Growth EC_{50} values for both Zn and Cu were found to be relatively low when compared to other studies i.e. 2.70 and 0.844 µg/g, respectively (Table 4.10 and Table 4.13). Bat and Raffaelli (1998) reported growth EC_{50} values of 31.66 µg/g Cu and 28.59 µg/g Zn for the estuarine amphipod *Corophium vollutator.* Roman *et al*. (2007) also reported exceptionally high growth EC_{50} values for *Hyalella azteca* (194 µg/g).

Tissue analysis was not conducted in the chronic toxicity experiment therefore, bioaccumulation of metals with increasing sediment concentration was not reported. McGee *et al*. (1998) reported that

neonates and juveniles of the amphipod *Leptocheirus plumulosus* showed reduced tolerance to metals. It is, therefore, not clear whether the lower number of offspring was due to a decrease in reproductive rate or a decrease in juvenile survival with increasing concentration as juveniles were only counted at the end of the exposure period. It is, however, clear that even at low concentrations, both Cu and Zn had a negative effect on fecundity and thus population dynamics of *M. zeylanica*. As *M. zeylanica* forms an integral component of the benthic food chain, contamination areas where *M. zeylanica* is in abundance could result in impacts on the health and dynamics of the population.

Following the 28 day Cu chronic exposure, the average increase in juvenile size decreased with increasing metal concentration, except for the 5th exposure (3 µg/g), in which juvenile growth was slightly higher (2.19 mm) (Table 4.15). The reason for the unusual juvenile growth in 3 µg/g is probably related to the fact that only one juvenile survived, as juvenile growth in all other Cu concentrations gradually decreased from the control through to the highest Cu concentration. Juvenile growth showed considerable variation within each concentration, suggesting that there may have been different size classes or cohorts of amphipods per exposure (Table 4.16). Excluding the 5th exposure, the control exposure showed both the highest number of juveniles as well as the most size classes, with the final exposure showing the lowest number of juveniles and number of size classes. These results suggest that both the highest success in fecundity as well as the highest reproductive success per adult amphipod were found in the control exposure, i.e. there were more amphipod generations (most size classes) within the 28 day control exposure, while those in the highest Cu concentration failed to reproduce past the first size class range (lowest size class).

Highest juvenile class sizes were recorded in the control exposure and decreased progressively with increasing concentration spiking (Table 4.16). These results, therefore, suggest that reproduction was most successful in the control experiment and that fecundity of amphipods decreased with increasing metal concentration. Green *et al.* (1999) and Costa *et al.* (1996) cautioned against interpreting growth data from chronic tests when there is significant reduction in adult survival, with the major concern being that growth may be influenced by the reduction in densities during the course of the experiment. This means that mortality will reduce densities of amphipods, resulting in more food available for surviving juveniles. The high adult survival in this study, thus suggests that density was not a confounding factor. In addition, analysis of covariance revealed that adult mortality did not significantly affect the fecundity results (Table 4.12).

According to Emery *et al*. (1996), sediment grain size may significantly limit amphipod growth and reproduction, as *Leptocheirus plumulosus* was severely stressed when clay or sand composed more than 75% of the sediment. Kennedy *et al.* (2009) also reported that grain size is one of the

main confounding factors affecting bioassay tests and that grain size may cause amphipod mortality due to an increase in stress when the species tolerance level is exceeded. Physical inspection of the exposure sediment in the current study indicated it was muddy sand i.e. neither clay nor sand were dominant in the sediment. It is, however, also important to note that no one species or test method is universally ideal for all environments, thus different species will prefer different sediment types. Teske and Wooldridge (2003) also emphasised the importance of sediment grain size in affecting benthic species composition as they found that, in South African estuaries, sediment type strongly influenced species distributions as opposed to estuaries in the Northern hemisphere, where salinity was found to be the major factor affecting species distribution. This result may suggest that *in situ* tests are more favourable as compared to laboratory tests as the organism is in its natural environment thus eliminating grain size as a confounding factor.

In situ tests, however, are difficult to control for environmental factors such as temperature, salinity, pH as well as predation. Laboratory tests, on the other hand, provide a constant environment for toxicity testing where all physico-chemical parameters can be controlled (Leverett and Thain 2013). Criticism of laboratory experiments however, include the relevance of the laboratory results in relation to the field.

McGee *et al*. (2004) argued, however, that laboratory results may be extrapolated to field populations as they found satisfactory agreement between the laboratory toxicological response of acute and chronic tests with the amphipod *L. plumulosus* and field results following their study of chronic sediment toxicity *in situ* and in the laboratory. Extrapolation of the results of this study, therefore, suggest that populations of *M. zeylanica* in the Mzingazi Canal may decline as a result of increased metal toxicants as reproduction and growth were adversely affected by increasing metal concentrations when tested in the laboratory. Such a decline in the Mzingazi canal may have negative impacts on nutrient cycling and predator populations as amphipods form an important part of the estuarine food-chain and therefore make an important contribution to the aquatic ecosystem. Mayer *et al*. (1995) reported that *L. plumulosus* is important in the cycling of nutrients through digestion of detritus and also stimulation of nitrification through sediment irrigation. Although behaviour of *M. zeylanica* was not considered in this study, it was observed to disturb the sediment throughout the experiment to such an extent that it made the removal of dead amphipods challenging as visibility was diminished by the disturbed sediment throughout the experiments. This may imply that *M. zeylanica* is important in stimulating nitrification and irrigation of sediment as it was in direct contact with the sediment.

5.4 Field validation

The validation test showed that amphipods responded positively to sediment with least metal contamination as survival was highest in least contaminated sites (Pelican Island, Bhizolo,

Manzimnyama) and lowest in more contaminated sites (RBCT, Mzingazi Canal and the Bulk (C7). A 72% amphipod survival was recorded at Pelican Island i.e. the site which showed least metal contamination, while only 40% survival was recorded in the Bulk (C7) area, which was the site that showed high concentration for most metals (Fig. 4.21). Mercury was high in the Mzingazi Canal, which resulted in high mortality of amphipods as Hg is amongst the most toxic metals to amphipods (Shuhaimi-Othman *et al.* 2011). This result confirms that *M. zeylanica* is sensitive to contaminated sediments and thus the field validation suggests that it is a suitable bioindicator of sediment metal contamination. Amphipods were collected higher up in the Mzingazi Canal up stream of the weir and as such they probably were not exposed to such high concentrations. This wil, however, have to be verified through further studies.

Although there is considerable literature on Richards Bay Harbour (Wepener and Vermeulen 2005; Greenfield *et al.* 2011; Wepener and Degger 2012), there are no data in the literature on metal concentrations in epi-benthic or benthic amphipods in KwaZulu-Natal estuaries. The one exception is the study by Vivier (2010) on *C. triaenonyx* and *G. lignorum* in the Nhlabane Estuary, in which the viability of these amphipods to be used in estuarine toxicity testing was assessed. There have, however, been extensive studies on amphipod bioaccumulation worldwide (Thomas 1993; Reish 1993; Cota *et al*. 1998, Lee *et al*. 2005; King *et. al*. 2006a; Onorati *et al.* 1999), indicating that South Africa is lagging behind in this field.

A comparison with ecologically relevant benthic amphipods suggests that Zn and Cu accumulation in *M. zeylanica* compared favourably with amphipods used elsewhere. Table 5.1 shows that accumulation was high for Cd, Ni and Pb. When compared to amphipods such as *G. lignorum*, *C. triaenonyx, P. excavatum*, *G. locusta* and *G. salinus, M. zeylanica* metal accumulation compared favourably for Zn, Cu, Ni and Pb. When compared to other *Melita* species such as *M. plumulosa* and *M. awa, M. zeylanica* tissue accumulation for both Cu and Zn was found to be rather low (Fig. 4.8 and 4.5). King *et al.* (2006a) reported concentrations of 470 and 330 µg/g for Cu and Zn respectively for *M. plumulosa* as well as 330 µg/g Cu and 370 µg/g Zn for *M. awa*. *Melita zeylanica* Zn accumulation, however, was found to be higher than that of *M. matilda* (140 µg/g). When compared to amphipods such as *G. oceanicus* and *G. setosus,* metal accumulation by *M. zeylanica* was also found to be relatively high. This result suggests that *M. zeylanica* is a suitable accumulator of metal and, therefore, it can potentially be used as a bench mark species for estuarine toxicity studies in South Africa.

Table 5. 1 Range of concentrations of metals (ug/g) recorded in amphipods of harbours during this and previous studies.

Species	Cd	Cu	Ni	Pb	Zn	Habitat	Reference
Melita zeylanica	26	59	36	11	227	Sand/Mud estuarine	This study
Grandidierella lignum	0.4	99	54	31	87	Sand/mud estuarine	Vivier 2010
Corophium triaenonyx	0.4	91	47	17	62	Sand/mud estuarine	Vivier 2010
Paracorophium excavatum		158-207				Sand/Mud estuarine	Marsden *et al*. 2013
Melita plumulosa		470 ± 240			390 ± 30		King 2006a
Melita matilda		120 ± 5			140 ± 8		King 2006a
Melita awa		330 ± 80			370 ± 20		King 2006a
Gammarus locusta		93-134	1.3-2.4	0.5	81-562	Intertidal rockey shore	Zauke *et al*. 2003
G. oceanicus	0.7-1.0	14-28		1.6-2.8	61-68	Sand/Mud estuarine	Zauke *et al*. 2003
G. salinus	0.1-0.5	75-135		2	62-91	Sand/Mud estuarine	Zauke *et al*. 2003

The Australian sediment quality guidelines for toxicity testing are commonly used in South Africa. Two values of importance are often reported with the Australian guidelines namely; the Effects Range Low (ERL) and Effects Range Medium (ERM). The ERL is the 10th percentile of the effects data of contaminants where adverse effects rarely occur and the ERM is the 50th percentile, where adverse effects frequently occur (ANZECC 2000). In this study Australian guidelines were applied for data comparison.

Sediment metal concentrations varied considerably amongst different sites sampled (Fig. 4.22.1 and Fig. 4.22), with the Bulk (C7), which is adjacent to the general bulk and metal ore terminal, showing highest concentrations for most metals. The Bulk Terminal is the biggest multipurpose and dry bulk terminal in South Africa (Turpie *et al.* 2002) and handles a variety of products ranging from chrome, manganese, titanium, ferro-fines through to woodchips. As it is a multipurpose terminal, in order to avoid inter-cargo contamination, all conveyor belts, rail trucks and vessel loaders need thorough washing after loading of a particular product, before commencing the next

product handling. This could account for the high sediment metal concentrations in the area. Sediment metal results showed that different metals were problematic in different areas of the harbour. At the Richards Bay Coal Terminal, sites RBCT (5) and RBCT (6), for instance, showed Zn concentrations as high as 508.07 and 541.43 µg/g, respectively.

Chromium, Cu and Zn were high in the Manzimnyama Canal with concentrations of 84.3, 36.9 and 302.6 µg/g, respectively. The Mzingazi Canal showed highest Hg concentration (11.08 µg/g), while Ni was high in the Small Craft Harbour (65.03 µg/g). Pelican Island showed overall low metal concentrations, while the Bulk (C7) terminal showed highest metal concentrations. This site showed concentrations of some metals several orders of magnitude higher than the rest of the sites (Table 5.2). For this reason, the Bulk (C7) results are a cause for concern and follow-up sampling will be required to assess the extent of the metal contamination in this area.

5.4.1 Aluminium and Iron

Iron and Al occur naturally in high concentrations in estuarine sediments (Vivier 2010), so those levels found in this study are not unexpected.

5.4.2 Copper

The Bulk Terminal is associated with loading and offloading of bulk material and ores, RBCT with coal export, while the Bhizolo and Manzimnyama Canals drain the industrial area behind the port and are more associated with runoff from industry. Bulk cargo handling at the Bulk (C7) would partially account for the high concentration of Cu at this site (434.31 µg/g), as ore spillage often occurs during loading/unloading of this cargo (Gracia *et al.* 2013). The high Cu concentration in the Bhizolo and Manzimnyama Canals could also be as a result of the surrounding industry as these systems are associated with their runoff. These canals are seen as an important and rare aquatic habitat in the port, not only are they fringed with mangroves, they also sustain a diverse community of fish and invertebrates and act as a nursery for various fish species of marine origin (Weerts and Cyrus 2002). This result is, thus, concerning as high concentrations of Cu can be accumulated by aquatic organisms within the harbour causing respiration difficulties and growth reduction (Kennish *et al.* 1992). Results of this study showed that, even though high concentrations were recorded at all sites, they did not exceed the ERM Australian guidelines for toxicity testing as used in South Africa (Table 5.2), except at the Bulk (C7) which showed a much higher Cu concentration (434.31 µg/g) than the ERM guidelines, while the Manzimyama Canal and Pelican Island showed values lower than the ERL values.

Table 5. 2 Mean sediment metal concentration at 9 different sites within Richards Bay Harbour with historical data and Australian standard guidelines as used in South Africa.

Sampling date		As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Reference
2012										
	Pelican Island	3.78	19.45	21.37	11.56	6.82	42.83	7.04	130.23	Current study
	Bhizolo Canal	5.34	34.35	47.18	87.2	3.77	10.45	7.31	107.61	
	Manzimnyama Canal	3.91	17.18	84.33	36.96	5.64	56.10	7.00	302.64	
	Bulk C7	6.92	38.20	54.07	434.31	3.93	121.68	9.15	1487.86	
	Bulk C4	4.06	18.95	55.85	131.17	4.72	45.85	8.29	354.15	
	Small Craft	5.03	37.18	37.66	84.75	4.18	65.03	7.85	570.41	
	RBCT 5	5.94	26.64	40.41	67.17	3.49	71.75	5.39	508.07	
	RBCT 6	4.51	25.20	43.45	80.42	4.04	79.39	10.00	541.43	
	Mzingazi Canal	5.79	4.68	56.55	10.78	11.08	34.82	4.67	94.74	
	Action Value S.A	30	1.5	50	50	0.5	50	100	150	
	Effects Range Low (ERL) Australia		1.2	81	43		21	46.7	150	
	Effects Range Medium (ERM) Australia		9.6	370	270		51.6	218	410	
1998				21-339.8	10.0-258				14-193.0	Archibald and Parsons (1998)
1996-1997				38.4-221.9	1.82-53.5				153-181	Wepener and Vermeulen (2005)
1985				114	25.0				53	Henry *et al.* (1989)
1972-1981				74.80	1.1-40.0				14-179	Hennig (1985)

5.4.3 Zinc

Zinc was also found to be one of the metals of concern at the Bulk (C7) terminal within the harbour. Sediment concentrations in the Manzinyama Canal, the Bulk (C4), Small Craft, RBCT (5), RBCT (6) and Bulk (C7) all showed elevated concentrations of 302 µg/g, 354 µg/g, 570 µg/g, 508 µg/g, 541 µg/g and 1487 µg/g, respectively (Table 5.2). These concentrations were much higher than those reported by Wepener and Vermeulen (2005), who reported concentrations ranging between 92.2 and 103.2 µg/g. The reason for these high Zn concentrations is not clear, although it is possible that there may have been a recent oil-spill at the time of sediment collection. Bu-Olayan and Subrahmanyan (1997) reported that Zn is one of the metals associated with oil spills and documented Zn concentrations of up to 1204.40 µg/g in organisms following the 1991 Gulf War oil spill. Only the Small Craft, RBCT (5), RBCT (6) and the Bulk (C7) showed concentrations exceeding ERM guidelines, with the remaining sites Bhizolo Canal, Pelican Island and Mzingazi Canal showing concentrations below the ERL guidelines. This result, therefore, raises concern as it implies severe contamination of Zn in parts of the harbour, which could prove deleterious to the estuarine fauna in the harbour. Although Zn is an essential metal for organisms, it can have deleterious effects at high concentrations. In some fish species, Zn has been reported to affect fertilisation and embryonic development of fish as well as in harpacticoid copepods (Ojaveer *et al*. 1981), thus potentially affecting the population dynamics of organisms associated with contaminated sediments. Although not initially part of the study, Polychlorinated Biphenyls (PCBs) and Poly Aromatic Hydrocarbons (PAHs) sediment analysis was conducted following the discovery of the high Zn concentration and concerns of potential oil-spills highlighted in this study (Fig. 4.23) and Fig. 4.25. As relatively low PCB and PAH concentrations have been previously reported in Richards Bay Harbour (Degger 2010), only the two sites showing the highest Zn concentration were analysed i.e. the Bulk (C7) and RBCT (5). Although concentrations were found to still be relatively low, high PAH molecular compounds as well as high Cl-ring congener compounds were present in Richards Bay Harbour. Further PCB and PAH studies, therefore need to be conducted in the Richards Bay Harbour to determine the extent of contamination as well as source of these organic compounds (See Appendix 1).

5.4.4 Cadmium

Cadmium background levels are generally quite low with sediment concentrations normally less than 0.2 µg/g (Bryan and Langston 1992). Cadmium was found to be amongst the chemicals of concern in Richards Bay Harbour as it occurred at exceptionally high levels at three sampling sites; Bhizolo Canal, the Bulk (C7) and Small Craft, with concentrations of

34.35 µg/g, 38.32 µg/g and 37.18 µg/g, respectively (Table 5.2). Rubenstein *et al.* (1983) reported sediment concentrations between 5-38 µg/g and regarded 38 µg/g as highly toxic. These results suggest that there is potential Cd contamination in Richards Bay Harbour, as Cd concentrations were comparable to concentrations regarded as highly contaminated. When considering the ERM guidelines, Cd was found to be problematic at all sites except the Mzingazi Canal, where concentrations were found to be lower than the recommended ERM guidelines. According to Butler and Timperley (1996), Cd is not an essential metal, therefore, even the smallest quantities can be deleterious to organisms, more so since Cd is reportedly the most toxic metal to organisms after Hg (Abel 1989). Most reported sources of cadmium contamination are sewage as well as industrial effluents (Abel 1989). Some of the observed toxic effects of Cd exposure include inhibited ability to utilise glucose (Shore *et al.* 1975) and impaired reproduction in crustaceans (Guidici and Guarhiho 1989). Luoma *et al.* (1990) also reported that high Cd concentration generally results in a population reduction of aquatic crustacean organisms.

5.4.5 Mercury

Mercury was found to be a metal of concern at all sample sites within Richards Bay Harbour as all sites exceeded the action value for South Africa by an order of magnitude. Mercury is a non-essential metal with no known nutritional function in the body (Butler and Timperley 1996) and according to Abel (1989), it is also the most toxic metal to organisms. Sediment levels generally range from 0.2 to above 3 µg/g (Alloway 1990). In this study, sediment Hg levels as high as 11.8 µg/g were recorded in the Mzingazi Canal. These elevated concentrations were not expected as the Mzingazi Canal is removed from industrial activity and, therefore, expected to have relatively low sediment metal concentrations. There is, however, a sewage maceration facility adjacent to the canal, and sewage spills have been reported on occasions over the past few years, which could explain the high Hg concentration in the area. High Hg content in aquatic systems is associated with sewage sludge and different methodologies have been developed to try and determine the amount of Hg in sewage sludge (Abel 1989). The flow injection mercury system (FIMS), for instance, is an integrated flow injection Hg cold vapour generation with a highly sensitive detector to measure Hg content within the sludge (www.perkinlmer.com, ICON 2001). High Hg concentrations have been reported to affect amphipod feeding in estuaries thus affecting population dynamics of amphipods within estuarine systems (Bundschuh *et al.* 2011). Read (2005) also reported that concentrations of 0.2 µg/g chronically affected growth, behaviour and reproduction of juvenile and adult fish. Mercury is thus a metal of concern within Richards Bay Harbour and could potentially affect population dynamics of organisms within

the harbour. Although Hg concentrations are reported to be very high in this study, this needs to be verified with further work using alternative analysis techniques.

5.4.6 Lead

Lead was found in relatively low concentrations within Richards Bay Harbour with concentrations ranging between 0.23-10 µg/g. No sampling site showed Pb concentrations above the ERM or ERL guidelines. In the UK, Pb concentrations ranging between 25 and 2700 µg/g were reported in estuaries (Bryan *et al*. 1980). Lead was, therefore, not found to be a metal of concern in Richards Bay Harbour in this study. In Canada even lower concentrations of Pb were recorded, ranging 0.01-1.15 µg/g (Bryan *et al.* 1980), meaning that these results do not necessarily imply the absence of Pb within the harbour.

5.4.7 Chromium

Another metal that was present at acceptable concentrations within the Harbour was Cr. None of the sampling sites exceeded the ERM guidelines for Cr. The Manzimnyama Canal (84 µg/g), however, did exceed the ERL guidelines and the Bulk (C7 & 4), as well as the Mzingazi Canal were above the Action Value S.A. guideline. Chromium concentrations were found to be relatively low compared to other studies, with Cr concentrations ranging between 200 and 800 µg/g in UK estuarine sediments and up to 3700 µg/g in Sawye's Bay, New Zealand (Bryan and Langston 1992).

5.4.8 Nickel

Nickel was found to be one of the metals of concern in Richards Bay Harbour. The Bhizolo Canal, Manzimnyama Canal, Small Craft, RBCT and the Bulk (C7) all showed concentrations higher than the ERM guideline which suggests that there is contamination of Ni within the harbour. The concentrations range for Ni was found to be between 43-121 µg/g, with the Mzingazi Canal showing the lowest concentration and the Bulk (C7) the highest. Nickel is considered to be one of the essential elements required by the body, as with Cu and Zn, however, it also rates high amongst the most toxic metals in sediment (Abel 1989).

5.4.9 Arsenic

Arsenic was not found to be a metal of concern as concentrations were found to be well below the Action Value S.A. guideline at all sites.

5.5 Tissue accumulation

Of most relevance in toxicity assessments is the proportion of metal in the sediment that is bioavailable in the exchangeable water and acid soluble fraction (Rainbow 2007, Re *et. al*.

2009). Coetzee (1993) and Thompson *et al*. (1984) highlighted that total sediment concentration of metals does not necessarily reflect their bioavailability and that sequential extraction reflects a better representation of metal availability. In this study the sequential extraction of the sediment was not conducted, however, Wepener and Vermeulen (2005) conducted sediment extraction for Richards Bay Harbour sediment. These authors found that all metals were readily bioavailable with Zn showing highest bioavailability.

In this study, the tissue accumulation pattern was similar to that of sediment metal concentrations within Richards Bay harbour i.e. Fe>Al>Zn>Cr>Cd>Ni>Cu>Hg>Pb>As (Fig. 4.24.1, Fig. 4.24.2). The similar concentration patterns for sediment and tissue bioassays further emphasises that *M. zeylanica* is a net accumulator of metal and, therefore, it can potentially be used as a South African benchmark species for estuarine toxicity testing in the country. This was also emphasised by the Pearson's correlation curve (Fig. 4.25.1, Fig. 4.25.2) which showed a positive correlation between sediment metal concentrations and the uptake of metal for all metals.

5.6 Conclusion

Lowest median (phi) as well as % mud in the sediment were recorded at Pelican Island and in the Mzingazi Canal (Fig. 4.20), these were also sites with lowest overall metal concentrations with the exception of Hg in the Mzingazi Canal (Table 5.2). This is in agreement with the literature as it is widely reported that highest metal concentrations are generally associated with sediment fines i.e. with high mud content (Hennig 1985; Chapman and Wang 2001; Rauret *et. al*. 1989; Vivier 2010).

The results of this study suggest that *M. zeylanica* has the ability to accumulate metals to high concentrations, which is one of the essential properties of a good indicator species. The most recent study on Richards Bay Harbour metal concentrations by Degger (2010), reported that metal concentrations are strongly influenced by spatial and seasonal variation and that there was a notable increase in metal concentration when compared to previous studies. Furthermore, Wepener and Vermeulen (2005) stated that metal concentrations tend to be higher in summer, during the high rainfall season, than in winter, due to high precipitation and the resultant increased storm water runoff. Although it is true that sediment samples in this study were collected in September and elevated sediment metal concentrations were expected in known contaminated areas, metal concentrations in some areas were much higher than expected, particularly for Zn. Even though the results of this study showed elevated metal concentrations when compared to the findings of Degger (2010), they were found to be lower than the ERM values for As, Cr and Pb as used in South

Africa. With the exception of the Bulk (C7), Cu concentrations were also found to be within the ERM guidelines (Table 5.2).

Field validation results from this study are significant as they imply that metal contamination is prevalent in different parts of the harbour and that different metals show elevated concentrations in different areas of the harbour. It is therefore, important that continuous monitoring of the harbour be conducted to ensure that the system remains in good health. It is also important to note that, even though Richard Bay Harbour is not a protected area, it is still a highly ecologically significant estuarine system. Together with the adjacent Mhlathuze Estuary, it maintains a very high biotic diversity and is regarded as ecologically one of the most important estuarine systems along the Zululand coastal region (Mzimela *et al.* 2003). It is, therefore, important to regularly monitor toxicity levels within the harbour to ensure that the ecological integrity of the system is protected.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

In a review of Cu and Zn toxicity publications over the past 10 years, it was found that various amphipods were used in different types of toxicity studies, however *C. volutator* was the amphipod most used in bioassays. *Corophium colo* was found to be the least sensitive to Zn and Cu showing the highest LC_{50} value and *Melita awa* was the most sensitive amphipod showing the lowest LC_{50} value for both Zn and Cu. With the exception of *M. matilda*, *Melita spp.* were reported to be the most sensitive amphipod genus, they would, therefore, make good bioassay test organisms as they are sensitive to toxicants and able to survive in different media.

One question which has been raised in estuarine research in South Africa is whether South Africa is keeping up with global trends in terms of estuarine toxicity research (Wepener and Degger 2012). It was found in the overview study that the top five active countries in estuarine bioassays are the US, China, UK, Germany and France. It was further noted in this overview, however, that Australia has also produced a considerable number of papers in the past 10 years, suggesting that South Africa is still lagging behind in estuarine ecotoxicology research with regards to global trends (Sus *et al*. 2012).

Chronic bioassays have received more attention in the past decade than in previous years; this finding is in agreement to that of Sun *et al.* (2012). Of the chronic endpoints utilized, fecundity was the most frequently reported. It was also found that the most frequently used period of exposure was 30 days. There was, however, a wide range of exposure periods ranging from 130hrs to 18 months. The shorter bioassays focused more on behaviour while longer ones reported on bioaccumulation. Ten day and 28 day studies were also frequently reported, according to the literature review in this study however, there is still need for longer bioassays incorporating life-history studies such as average number of broods per female and life-cycle of the amphipod, as these give a more holistic interpretation of bioassays. One of the most noted challenges in this review was the difficulty within inter-laboratory comparisons, which was attributed to various factors including bioassay methodology, weight, gender and adaptation. Even though standardised methodologies are applied, there was still considerable adjustment of these methodologies needed to fit specific studies. There is therefore, need to ensure that flexibility in method modification is limited, particularly with physico-chemical parameters such as salinity and temperature, as these are known to affect organism behaviour more profoundly.

During exposure tests undertaken for this study, the 10 day bioassay revealed that *M.*

zeylanica is equitably sensitive to Cu and Zn at lower concentrations (LC_{50}) and more sensitive to Cu at higher concentrations (LC_{90}) and demonstrated metal accumulation during both Zn and Cu bioassays. *Melita zeylanica* also demonstrated a strongly positive correlation between sediment and tissue concentration during the 10 day bioassay. When compared to other *Melita* sp., such as *M. matilda* and *M. awa* (King *et al.* 2006a), *M. zeylanica* was found to be more sensitive to both Cu and Zn, showing lower LC_{50} values than both these amphipods. Furthermore, *M. zeylanica* was found to accumulate both Cu and Zn to higher concentrations than *M. matilda* (King *et al*. 2006a). Results of this study, therefore, suggest that *M. zeylanica* can potentially be used as test organisms during acute toxicity testing for contaminated sediment, notably Zn and Cu, in subtropical estuaries meaning that the first hypothesis was accepted.

Fecundity, juvenile body length and the number of size classes decreased with increasing sediment metal concentrations, with fecundity showing a negative correlation with both Zn and Cu during the 28 day bioassay in this study, meaning that increasing metal concentration of both metals inhibited fecundity for *M. zeylanica*. Body length was also found to be negatively correlated with increasing Cu concentration following a 28 day Cu bioassay. According to these results, growth and fecundity are suitable endpoints in chronic toxicity testing with *M. zeylanica* as both were negatively affected by increasing metal concentrations

The 96hr salinity test showed that *M. zeylanica* is tolerant to a wide range of salinity suggesting that hypothesis 4 was accepted. The high ammonia LC50 value following 96hr exposure indicated that hypothesi 3 was rejected.accepted.

Analysis of sediment from Richards Bay Harbour showed that sites with the highest mud and organic concentration also showed highest metal concentration. This result is in agreement with reports by various authors (Zhou *et al*. 2003, Halconson *et al.* 2004, Wepener and Vermeulen 2005), that high metal concentrations are prevalent in fine sediment with high particulate matter. Arsenic, Cr and Pb, were found to be less than the ERL and Action Value S.A. guidelines for all sample sites in this study. This, however, by no means suggests an absence of these metals within the harbour as, for instance, Cr was found to be higher than concentrations reported elsewhere in the country (Henry *et al.* 1989).

Highest metal concentrations were found at Bulk (C7), RBCT (5) and RBCT (6). This finding was in agreement with Wepener and Vermeulen (2005) who also reported high metal concentrations at nearby sites. Results of the current study, however, also indicate that different metals are problematic in varying parts of the harbour. Zinc, for instance, was a metal of concern at Bulk (C7), Small Craft, as well as RBCT (5) and RBCT (6); while Ni was found to be a metal of concern in the Manzimnyama Canal. Metals of concern at almost all

sites were Cd, Ni, Zn and Hg, with Hg showing highest concentrations in the Mzingazi Canal. The high Hg concentration in the Mzingazi Canal was not expected as this site is removed from industrial activities and has not been historically reported as an area of concern in terms of metal contamination. Although the Mzingazi Canal was previously reported as relatively uncontaminated (Wepener and Vermeulen 2005), data from this study suggest it not to be the case, with very high Hg concentrations being recorded. These elevated Hg concentrations will need to be taken into account in future toxicity research and will need to be monitored. Greenfield *et al.* (2014) reported high accumulation of Zn and Ni in the mussel *Perna perna* following a 6 week active biomonitoring study in Richards Bay Harbour. This result indicated that there is metal contamination within the harbour, and that organisms dependent on the harbour as a habitat may be adversely affected by these metals. The Bulk (C7) site was found to be of most concern as it had the highest concentrations for most metals. These were above ERM guidelines for all metals excluding As, Cr and Pb. Weperner and Vermeulen (2005) also indicated this site as showing highest metal concentration, indicating that the Bulk (C7) is an area of concern within Richards Bay Harbour which requires urgent monitoring.

Field validation results suggested that *M. zeylanica* is a net accumulator of metals, with Hg and Cr showing higher accumulation when compared to other metals. Following exposure to Richards Bay sediment, it is concluded that *M. zeylanica* is sensitive to sediment metal concentrations in this harbour. Results from this study showed that the use of *M. zeylanica* as an estuarine toxicity test species in South Africa has potential application value for implementation within a national monitoring framework in a manner similar to that used for freshwater systems. As such, the 5th and 6th hypotheses were both accepted. Results of this study also indicated that *M. zeylanica* is able to accumulate metals to high concentrations comparable to those currently used in toxicity testing elsewhere. *Gammarus locusta,* for instance was reported to accumulate Zn concentrations ranging between 81 - 562 (Zauke *et al*. 2003), while, in this study, a range between 89 - 227 µg/g was recorded in *M. zeylanica*. Zauke *et al.* (2003) also reported a Cu accumulation range between 14 -28 µg/g in *Gammarus oceanicus,* while a range between 11-59 µg/g Cu was recorded in this study. It was also found in this study that Cu tissue accumulation following the 10 day Cu acute toxicity test ranged between 51-100 µg/g, which was comparable to that of the closely related amphipod *M. matilda,* with Cu recorded accumulation of 120 ± 5 µg/g (King *et al.* 2006a). It can, therefore, be concluded that *M. zeylanica* is a good accumulator of metal with accumulation tendencies comparable to those of amphipods used in toxicity testing elsewhere.

Richards Bay Harbour is both ecologically and economically of high importance as it serves

as a fully functional estuarine ecosystem as well as the largest cargo port in South Africa (Turpie *et al.* 2002). It is therefore important that management of the harbour incorporate both these vastly divergent responsibilities of the harbour. There have recently been proposals to expand Richards Bay Harbour over the next 40 years to increase its bulk cargo and container handling capacity (CSIR 2005). It is important to note that as the development of the harbour is associated with excessive dredging, re-suspension of metals in the sediment is inevitable, thus secondary metal contamination within the harbour will be prevalent and this will impact adversely on organisms dependent on the harbour ecosystem. It is evident that future development will potentially have a considerable negative impact on the role the harbour plays on the estuarine ecosystem. A management plan should (if not already included) include components that would reduce any significant losses to the estuarine ecosystem and ecosystem functioning.

The National Environmental Management: Integrated Coastal Management Act (Act No. 24 of 2008) requires estuaries of the Republic of South Africa to be managed in a co-ordinated and efficient manner. In accordance with a National Estuarine Management Protocol, Estuarine Management Plans (EMPs) are required to be drawn for all South African estuaries to ensure sustainable estuarine resource utilisation. Although Transet National Ports Authority (TNPA) have conducted long-term studies on metal toxicity within Richards Bay Harbour, these studies have mostly focused on water quality and not on sediment toxicity, furthermore, there is currently no EMP for Richards Bay Harbour. This is of great concern, as owing to its strategic locality and vastly diverse economic and ecological roles, a large number of different stakeholders (national, provincial and regional, government, industries, individuals and ecologists) all have an interest in the sustainable functioning of the harbour.

There is, therefore, an urgent need for an EMP to be drawn up and implemented for Richards Bay Harbour as it will serve to maintain harmony between the stakeholders concerned. The fact that no EMP has been designed and implemented in Richards Bay Harbour since the development of the harbour 27 years ago, is of concern. It is, however, encouraging to note that the Department of Water and Sanitation has recently published a ministerial declaration following a 2014 Gender-Water Conference held in the Eastern Cape Province (www.unesco.org), which states that ministers commit to follow the guidelines of the UNESCO World Water Assessment Program 2016, which recognises the importance of morphodynamics and integrated estuarine systems management.

Turpie *et al.* (2011) conducted biodiversity survey for estuaries in South Africa in order to establish which estuaries should be assigned estuarine protected area (EPA) status,

following the National Water Resource Classification System (Dollar *et al.* 2010). These authors classified Richards Bay Harbour as currently in a C-class health category and recommended that it should be improved to A-class or BAS (Best Attainable State meaning) that Richards Bay should be restored to its best possible health state.

The National Water Act states that no estuary should be graded under D-class. This is of high concern for Richards Bay Harbour as the proposed development of the harbour will most likely cause its ecological deterioration. An EMP is thus a key-factor for the future conservation of Richards Bay Harbour. As part of the EMP, it is imperative that a biomonitoring program be designed using a suite of ecological tools such as acute and chronic amphipod toxicity tests based on sensitive local species such as *M. zeylanica*.

6.2 RECOMMENDATIONS:

I. Inter-laboratory comparison is difficult as different studies use different methodologies under differing physico-chemical parameters. Laboratory based toxicity studies, should therefore be more standardised to allow international comparison of results.

II. Although there has been evident growth in chronic bioassay studies internationally in the past 10 years, acute tests are still extensively conducted, while life-history studies incorporating life-cycle experiments are still not well covered in bioassays. More chronic and life-history bioassays, therefore, still need to be prioritised in the future as an integral part of biomonitoring studies as these provide a more holistic understanding of ecotoxicological issues.

III. *Melita zeylanica* should be considered as a toxicity benchmark species in estuarine toxicity studies in South Africa. More studies under standardised laboratory conditions should be conducted to further validate *M. zeylanica* as a benchmark toxicity test species in South Africa.

IV. More organic and inorganic amphipod toxicity bioassays should be conducted in South Africa to allow national, inter-laboratory comparisons which will lead to more reliable results based on scientific studies.

V. This study did not attempt to describe sources of metal contamination within Richards Bay Harbour; however, it was evident that spatial trends exist with regard to the magnitude and frequency of metal contamination there. Further studies are required for finer scale identification of areas of metal contamination of concern. Potential anthropogenic impact areas should, therefore, be identified to enable monitoring and management of these metal contaminants.

VI. Although there have been reports that procedures to identify and control sources of metal contamination within Richards Bay Harbour have been put in place, the high metal concentration, particularly, for Zn, Cd, Hg and Cu, show that these procedures are either not efficiently implemented or ineffective. More attention has to be dedicated in ensuring proper implementation of monitoring procedures and, thus to enable possible modification where necessary.

VII. Richards Bay Harbour is nationally recognised as an estuarine ecosystem of high conservation importance. The development of an Estuarine Management Plan for the port, within the framework of the National Estuarine Management Protocol, should be regarded as high priority, given the current toxicity issues identified and the probable implication of the future expansion of port activities.

VIII. International water and sediment quality guidelines are currently being used in South Africa. Different geographical areas, however, have unique environmental characteristics suitable for organisms inhabiting a specific area, which makes, to a certain extent, the use of international guidelines inappropriate for validation of bioassays in different environments. National Sediment Quality guidelines should, therefore, be developed for organic and inorganic contaminants to allow bioassay interpretation using endemic organisms.

IX. With the global economy fast becoming bio-based and continued emphasis on 'going green', it is becoming even more important for South Africa to utilise its natural resources more efficiently and sustainably. This country has to recognise the import role of estuarine ecosystems as they form a vital link between marine and freshwater systems. It is also important to realise that, as water is essential to life and is the very

essence of sustaining a ‘green’ environment, water is a scarce natural resource in South Africa. It is, therefore, important to incorporate scientific findings in water management plans to ensure that water is sustainably and efficiently utilised in the country.

Chapter 7

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Appendix 1

PCB & PAH ANALYSIS

Estimation of ecotoxicological risk to exposure of organic chemicals in sediments of Richards bay Harbour through sediment toxicity experiments and comparison with Sediment Quality Guidelines (SQGs)

A.1 Introduction

High sediment concentrations of Zn have been previously associated with potential oil spills (Gohlke *et al.* 2011), the unexpectedly high concentrations found in the harbour sparked concern of possible oil spill since the harbour is highly characterised by shipping activities. To eliminate these concerns, Polychlorinated Biphenyls (PCBs) and Poly Aromatic Hydrocarbons (PAHs) sediment analysis was conducted although not initially part of this thesis. As such this section is added as the appendix. Because low PAH and PCB concentrations have been historically reported in Richards Bay Harrbour (Degger 2010), sediment from only two sample sites with highest concentration was analysed, i.e. RBCT (5) and Bulk (C7).

The aim of this section of the study was to determine whether organic compound contamination is a potential concern within Richards Bay Harbour.

A.2 Materials and methods

A.2.1 Sediment analysis

Freeze dried sediments (5–10 g) were placed in cellulose thimbles (pre-cleaned by Soxhlet extraction), and extracted using dichloromethane (DCM) for 18 h. Each DCM extract was concentrated using a rotary evaporator and cleaned up on a florisil column. The target chemicals were eluted with hexane elution (100 ml). Extracts were percolated through activated copper to remove sulphur, subjected to sulphuric acid clean-up and exchanged into hexane solvent. Final extracts were reduced to 100 µl under a gentle nitrogen stream.

Samples were analysed by gas chromatography with a Perkin Elmer Autosystem gas chromatograph fitted with a ^{63}Ni electron capture detector. The capillary column, SBP-5 (Supelco), was 30 m long, 0.25 mm i.d. with a 0.25 µm thick coating. PCBs were quantified using external standard mixture Aroclor 1260 purchased by Supelco Inc., USA. The samples were confirmed for 28 PCB (IUPAC numbers: PCB; 3Cl: -28 + 37, 4Cl: -52, 5Cl: -95, -99,101 105, -110, 114, 118 and 123 6Cl: -128, 138, 141, 146, and 149 7Cl: -151, 153, 156, 157, 167, 177 170, 180, 183, 187, 189 and 10Cl:- 209), with a GCQ plus ion trap mass spectrometer (MS) from ThermoFinnigan in selected ion monitoring mode (SIM). A trace gas chromatograph was equipped with an AS 2000 autosampler (ThermoFinnigan) and fitted with a Rtx-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm) from Restek. GC conditions and information on target/qualifier ions are described

elsewhere Pozo *et al*. 2012; Pozo *et al.* 2013). All compounds were analytical standards of >99% purity.

A.2.2 Quality assurance/quality control (QA/QC)

The procedures described above were checked for recoveries and reproducibility. Procedural blanks and reference material (purchased from National Institute of Standards and Technology (NIST)), were analysed for QA/QC purposes. Prior to sediment extraction, six analytical blanks were prepared using the same extraction and clean-up procedure. A solvent blank was analysed every 15 samples to check the response of the gas chromatograph (GC). A recovery standard was also evaluated by spiking sediment samples with PCB-30, with an average recovery >80%. Concentrations were recovery corrected. Analysis of the NIST reference material for sediments (HRM-1939A) showed a mean PCB recovery of 93.5%. PCBs -70 + 76, 95, -60 + 56 and -180 had significant blank interference. Limit of detection (LOD) was defined as the average blank ($n = 4$) plus thee standard deviations (SD). When target compounds were not detected in blanks, 2/3 of the instrumental detection limit was used as the Method Detection Limit (MDL). All qualified data (i.e. exceeding the MDL) has been blank corrected. The MDL was approximately 0.05 ng ml^{-1} for most individual components. Reported concentrations were adjusted by subtracting blank values and calculated on ng g^{-1} dry weight (d.w.) basis.

A.3.1 SQG Results

Potential ecotoxicological risk evaluation was also estimated. SQG calculations such as Effects Range Low (ERL) and Effects Range Median (ERM), as adopted from McDonald (1996), were applied to evaluate sediment quality in relation to PAH levels in the Richards Bay Harbour. Table (A1) presents the concentration ranges of PAHs proposed by international SQGs (ERL–ERM) (McDonald 1996) and the two sampling points at Richards Bay Harbour. SQG values showed that both PAH's detected in this study were below the ERL standards for total PAH's (99,41 ng.g-1 d.w. and 74,07 ng.g-1 d.w. for RBCT (5) and the Bulk (C7), respectively). Neither of the two samples were between the ERL and ERM nor above the ERM (Table A2).

A.3 Results and Discussion

Concentration of PAH's ranged from 35.65 to 58.94 $ng.g^{-1}$ d.w (Table A2.The two PAH compounds detected in this study were higher molecular weight PAH's as characterized by higher number of ring chains.

Dibenz[*a*,*h*]anthracene is a five-ring organic compound with the chemical formula $C_{22}H_{14}$ (Nieuwoudt *et al*. 2011). Dibenz[a,h]anthracene is classified as a heavy PAH's by the US EPA with the enthalpy of fusion ranging between 26.8 and 31.2 24.5 (Fu and Suuberg 2011).

Benzo[*ghi*]perylen is a six-ring organic compound with the chemical formula $C_{22}H_{12}$ and is classified as a priority pollutant by the US EPA with the enthalpy of fusion range between 16.5 and 18.3 16.3 (Fu and Suuberg 2011), this PAH was the highest detected compound in both sample sites in this study. Total PAH concentration was higher in RBCT (99,41 ng.g-1 d.w) than the Bulk (C7) (74,07 ng.g-1 d.w) (Table A1).

PCB levels were higher in the Bulk (C7) than in RBCT (5) (Table A3), total PCB concentrations were 3.77 and 5.88 ng.g^{-1} d.w. for RBCT and the Bulk (C7) respectively (Fig. A1, this result was an inverse of the PAH findings, which were found to be higher in RBCT (5) (Table A1). The highest percentage of PBCs were found to be the 10-Cl PCB congeners (Fig. A2).

Table A 1 SQGs values for PAH and number of stations amongst ranges of international Sediment Quality Guidelines at the Richards Bay Harbour.

Compound	SQG (ERL - ERM)(ng g^{-1})	No of stations		
		<ERL	ERL-ERM	>ERM
Ace	16-500	N/F	N/F	N/F
Flu	N/A	N/F-	N/F	N/F
Phe	240-1500	N/F	N/F	N/F
Ant	N/A	N/F	N/F	N/F
Fl	19-540	N/F	N/F	N/F
Pyr	665-2660	N/F	N/F	N/F
BaA	261-1600	N/F	N/F	N/F
Chr	384-2800	N/F	N/F	N/F
BbF	N/F	N/F	N/F	N/F
D(ah)a	63-260	RBCT (5), Bulk (C7)		
B(ghi(p	-	-	-	-

N/F: Not Found

Table A 2 Concentrations (ng/g dw) of 16 priority polycuclic aromantic hydrocarbons (PAHs) in the Richards Bay Harbour.

	RBCT (5)	Bulk (C7)
Acy	N/F	N/F
Ace	N/F	N/F
Flu	N/F	N/F
Phe	N/F	N/F
Ant	N/F	N/F
Fl	N/F	N/F
Pyr	N/F	N/F
BaA	N/F	N/F
Chr	N/F	N/F
BbF	N/F	N/F
D(ah)a	40.47	38.42
B(ghi(p	58.94	35,65
Total PAH's	99.41	74.07
Mean PAH's	49.71	37.04
SD	13.06	1.96

Table A 3 PCB concentration per site in ng/g dw at the Richards Bay Harbour

Congeners IUPAC	RBCT (5)	Bulk (C7)
28.00	0.07	0.17
37.00	N/F	N/F
52.00	0.06	0.12
95.00	N/F	0.04
99.00	N/F	0.19
101.00	0.10	0.08
105.00	N/F	N/F
110.00	N/F	N/F
114.00	N/F	N/F
118.00	N/F	N/F
123.00	N/F	N/F
128.00	0.76	1.29
138.00	0.23	0.23
141.00	N/F	N/F
146.00	0.06	0.04
149.00	0.26	0.11
151.00	0.10	0.05
153.00	0.04	0.04
156.00	0.50	0.48
157.00	N/F	N/F
167.00	N/F	N/F
177.00	0.39	0.20
170.00	1.34	0.53
180.00	0.09	0.15
183.00	N/F	N/F
187.00	N/F	N/F
189.00	1.77	1.08
209.00	2.36	4.69

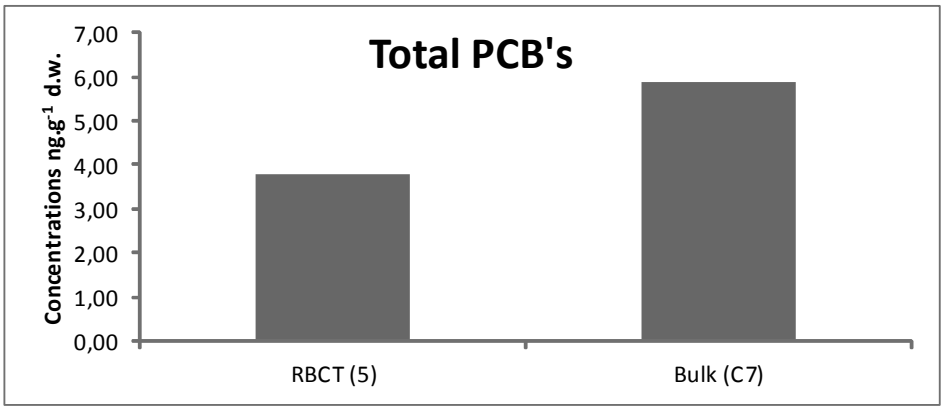

Figure A 1 Concentrations (ng/g dw) of PCB's from the Richards Bay Harbour.

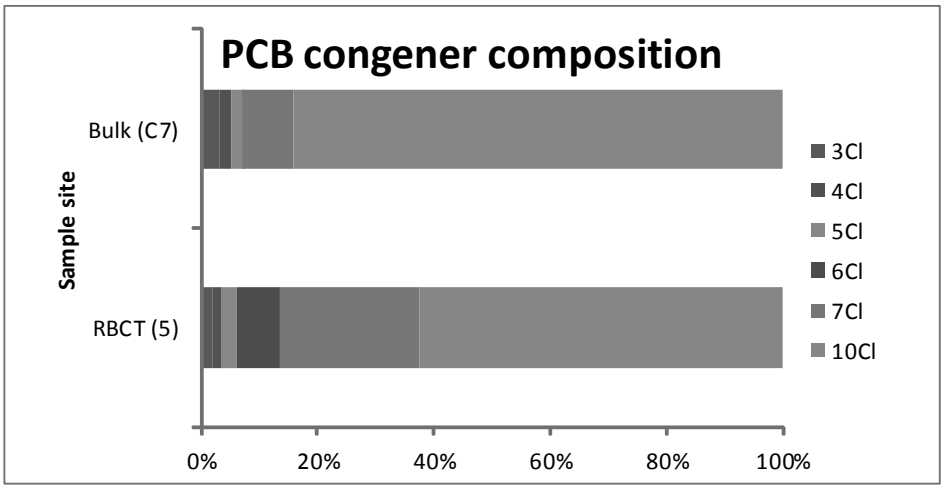

Figure A 2 PCB homologue composition % in sediment from the Richards Bay Harbour.

A.4 Conclusion

Even though concentrations are low for both PAH's and PCB's, high molecular weight PAH's as well as 10Cl PCB congener composition compounds were present. These results suggest that there is cause for concern with regards to possible oil-spills within Richards Bay Harbour. According to (Nieuwoudt *et al*. 2011), higher ring PAH's tend to accumulate and stay in the environment for prolonged periods as they are highly persistent and, therefore, are not easily eradicated (Pozo *et al*. 2012; Pozo *et al*. 2013). These heavy PAH's are also of pyrogenic origin, which means that they originate from intense heat such as burning of biofuels; i.e. coal and wood (Latimer *et al.* 1990). Extensive follow up experiments on PAH's and PCB's will, however, have to be conducted to determine the extent of PAH and PCB contamination within the Richards Bay Harbour.